THE EOACANTHOCEPHALA OF NORTH AMERICA, INCLUDING THE DESCRIPTION OF *EOCOLLIS ARCANUS,* NEW GENUS AND NEW SPECIES, SUPERFICIALLY RESEMBLING THE GENUS *POMPHORHYNCHUS*

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Evolution and adaptation in the ACANTHOCEPHALA have often been accompanied by the molding of structures which are superficially similar in the adults although they are derived from entirely different parts of the larvae. This tendency toward parallelism is all the more striking when it is recalled that complete adaptation to the influence of parasitism has been accompanied by extreme simplification of the body in all ACANTHOCEPHALA with a corresponding suppression of the number of avenues along which morphological diversity may be expressed. As an example of the foregoing statement, the superficially similar holdfast organs in *Filicollis* and certain species of *Polymorphus* may be cited. In the genus *Filicollis* it has long been recognized that the inflated holdfast organ is a conspicuous secondary modification to supplement the functions of the proboscis hooks in assuring firm attachment of the worm within the intestine of its host. Morphologically this modified structure had been very commonly regarded as an integral part of the proboscis until the writer (Van Cleave, 1947) assembled the evidences which prove it to be a modified region of the neck. The true proboscis in *Filicollis* is restricted to the small apical zone bearing the meridionally arranged hooks. In extreme contrast to this condition, the inflated bulb of *Polymorphus sphaerocephalus, P. kenti* and *P. altmani* does not involve any part of the neck but is directly the equivalent of the proboscis. This interpretation made possible for the first time a morphologically sound basis for differentiating between the genera *Filicollis* and *Polymorphus.* The species of both genera had been very generally confused in the taxonomic literature until a fundamentally sound morphological distinction was drawn between the two superficially similar, swollen holdfast organs.

In materials which have been in the writer's hands for many years a similar instance of confusing parallels is present. One object of this paper is to describe a new genus of the EOACANTHOCEPHALA based upon a previously unknown species with features superficially resembling the genus *Pomphorhynchus* (PALAEACANTHOCEPHALA). Morphological study of the new species reveals the fact that the points of resemblance have no direct phylogenetic value but are rather to be explained as a parallelism similar to that found in *Filicollis* and *Polymorphus.* The structures involved are wholly distinct one from the other since they belong to entirely different body regions.

In 1935, Dr. M. S. Ferguson secured a series of 11 acanthocephalans from *Lepomis macrochirus* Raf. (the bluegill) taken from the Ohio River at Shawnee-town, Illinois. In life, as well as after preservation, these worms are characterized by the possession of an enlarged bulb (Figs. 1, 5) between the proboscis and a long, narrowly cylindrical structure anterior to the main part of the trunk. Among the ACANTHOCEPHALA recorded for fishes, the genus *Pomphorhynchus* has been the only one recognized as possessing such structures. Furthermore, the deeply embedded proboscis and bulbular expansion within the tissues of the host intestine enhance the superficial resemblance between the newly discovered forms and specimens be-longing to the genus *Pomphorhynchus*. Only after specimens had been stained and mounted did details of morphology become evident which would emphatically deny any close degree of relationship between the worms from the bluegill and members of the genus *Pomphorhynchus*. The most striking of these differences (Text Fig. A) concern the nuclei of the subcuticula, the cement gland of the male, the form of the proboscis and its armature, the histological structure of the enlarged bulb and the cylindrical region between the body proper and the bulb. Because some details of structure in the original material were not readily determinable, the specimens were put aside in the hope that supplementary material might become available for study.

Two years later (1937) Mr. James S. Tucker made a small collection of ACAN-THOCEPHALA from *Pomoxis nigro-maculatus* (Le Sueur) (the black crappie) from Horseshoe Lake, near Cairo, in Southern Illinois. In this collection were 8 indi-viduals of the same species as the ones from the bluegill of the Ohio River.

Preliminary study of the two lots of material provided incontestable evidence that they represent an undescribed species of a previously unrecognized genus. Un-fortunately, no single individual is perfect enough to provide all of the morphological characters essential for detailed description and generic diagnosis. However, the truly distinctive features of the genus and species are unmistakably observable in all of the specimens so that there can be no possibility but that the material represents a single, well defined, species. The greatest difficulty in interpretation concerns the specific details of the proboscis and its armature. Even here there are no conflicting evidences, although mutilation has effaced some of the details on many individuals. The proboscis hooks are relatively weak and are apparently easily damaged when the deeply embedded proboscis and the enlarged bulb are removed from the host in-testine. Furthermore, the proboscis itself is relatively small and details of its arma-ture are rendered indistinct by the heavy wall of the bulb which surrounds the base of the praesoma. This is particularly true since the proboscis rarely protrudes far enough from the bulb to permit of full visibility. In most of the specimens, the proboscis must be viewed, at least in part, projected against the wall of the bulb and in several instances only an end view of the proboscis is available.

For 12 years this material has reposed in the writer's research collection of slides in the expectation that other and possibly more favorable material might be obtained. In the meantime, the writer has critically examined the records of numer-ous autopsies of both sunfishes and crappies from other habitats. These records include many host examinations of species from the Illinois River, Mississippi River, Rock River, and other streams and lakes in Illinois, Iowa, Wisconsin, Minnesota, Michigan, and New York but none of them gave any evidence of the presence of the new species. It seems probable that the species might be rather strictly confined in
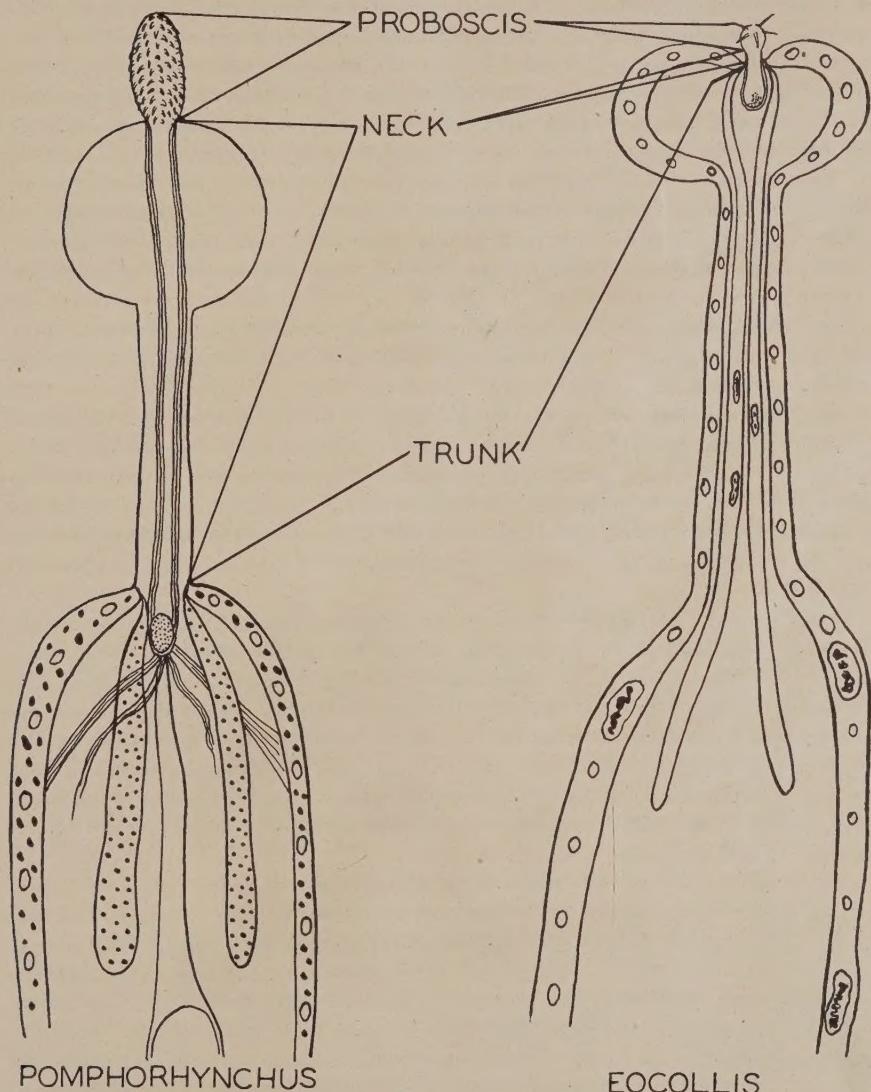
TEXT FIGURE A. Schematic diagram to show superficial resemblances between *Pomphorhynchus* (PALAEACANTHOCEPHALA) and *Eocollis* (EOACANTHOCEPHALA). The neck in *Pomphorhynchus* comprises a narrow cylindrical region which terminates distally in a thin-walled, inflated, bulb. Structures of similar form in *Eocollis* are modifications of the trunk while the neck is reduced to a mere vestige. In both genera the circular canals of the lacunar system are restricted to the trunk region where they are shown schematically as open circles in the body wall. The few large nuclei in the lemnisci and the wall of the trunk in *Eocollis* are replaced in *Pomphorhynchus* by numerous small nuclear fragments shown as black dots. The receptacle attached to the base of the proboscis is long and double-walled in *Pomphorhynchus,* short and single-walled in *Eocollis.*

its geographical distribution. The fauna of southern Illinois has much in common with that of Reelfoot Lake in Tennessee but Bangham and Venard (1942) in reporting on the parasites of the fishes of that lake record no individuals which could be mistaken for the new species. Nor do Bangham's records (1940) of the parasites of fresh water fishes of Florida carry evidence of any forms which might be misidentifications of the new species. In the two references cited, there is no record of the appearance of *Pomphorhynchus* in either bluegill or crappie and no mention of unidentified acanthocephalans in either host. Various students of the parasites of fishes in the lower Mississippi valley have made their collections of ACANTHOCEPHALA and records available to the writer but these likewise fail to give further evidence of the occurrence of the new species.

Failure to locate additional sources of material has led to the presentation of a description based on the available material. The form is here described as *Eocollis arcanus,* genus and species new.

In some immature individuals, the bulb behind the proboscis is not developed (Fig. 2) and in some fully mature individuals instead of a well formed bulb there is a series of rather large, irregular excrescences (Fig. 6) at the base of the proboscis. The former of these conditions is strikingly similar to those found in young adults of *Pomphorhynchus* which often lack the bulb. It was in young individuals of this sort that the nature of the "neck-like" region and bulb of *Eocollis* was first correctly interpreted.

Microscopic examination of the attenuated region and bulb in fully developed specimens reveals the fact that both of these structures are modifications of the front part of the trunk (Fig. 5) and do not represent morphological modifications of the neck as in *Pomphorhynchus*. For definiteness of reference and to avoid confusion in use of terms previously employed, the term "trunk bulb" is proposed for the trunk enlargement immediately posterior to the proboscis of *Eocollis*. Similarly, for the narrow, cylindrical portion of the trunk between the body proper and the body bulb, the name "false neck" is proposed to distinguish this region from the true neck in other acanthocephalans.

In *Pomphorhynchus,* and in all other genera with a well defined neck, the structure of its wall is conspicuously different from that of the body. As a part of the praesoma it is much thinner and lacks the circular vessels of the lacunar system characteristic of the trunk. Recognizing the morphological differentiation between neck and trunk, Rauther (1930) and more recently Lundström (1942) have given very satisfactory analyses of the distinction between these body regions. Rauther considered the neck as a region more fundamentally associated with the proboscis than with the trunk. In contrast, many writers have seemed to think of the neck as a more or less indefinite zone of transition between trunk and proboscis. In emphasizing his view, Rauther considered the neck and proboscis so fundamentally associated that he applied the name praesoma to the two regions combined. He further mentioned, as emphasized by Lundström, that a cuticular fold encircles the body to form a definite boundary between neck and trunk. This fold is an actual partition separating the subcuticular layer of the trunk from that of the neck. In *Eocollis* the cuticular fold or collar is entirely obscured by the heavy foldings of the wall of the trunk in the region of the trunk bulb.

In *Pomphorhynchus,* the boundary between trunk and neck is likewise made evi-

dent by the fact that the lemnisci have their origin at the boundary between these two zones and except for their points of origin they lie wholly within the trunk region. This is entirely different from conditions encountered in *Eocollis* where the lemnisci arise at the base of the neck (Text Fig. A) and extend backward from this line of insertion through the trunk bulb into the narrowed false neck of the trunk. This condition shows especially well in young individuals (Fig. 2) lacking the body bulb.

Furthermore, (Fig. 5), in fully mature specimens of *Eocollis,* the trunk bulb has its wall prominently encircled by the circular vessels of the lacunar system (Text Fig. A). In fact these vessels are just as conspicuous in the bulb as they are in the main portion of the trunk.

The parellel in appearance between the neck and neck bulb of *Pomphorhynchus* and the false neck and trunk bulb of *Eocollis* furnishes a striking example of the plasticity in body form and structure in the ACANTHOCEPHALA. Similar conditions in method of attachment within the tissues of the host intestine and general response to environmental conditions seem to have molded two entirely different sets of structures into a state of confusing similarity. The parallel in these structures seems to reflect a high degree of functional and morphological convergence between materials of entirely different embryological origin and histological construction.

Eocollis new genus

With the characteristics of the class (originally order) EOACANTHOCEPHALA as diagnosed by Van Cleave, 1936 and of the family Neoechinorhynchidae as outlined by Van Cleave, 1919. Anterior body extremity prolonged into a narrow, cylindrical, false neck which'in the region immediately posterior to the proboscis is usually inflated into a trunk bulb or a series of irregular excrescences. Proboscis short, cylindrical to globular, armed with three circles of six hooks each. The six giant subcuticular nuclei distinctive of the family are all restricted to the trunk region posterior to the trunk bulb and false neck. Body proper somewhat swollen posterior to the false neck with the single ventral and anterior one of the dorsal giant nuclei in the enlarged portion. Trunk tapering fairly rapidly toward posterior extremity. Lemnisci relatively 'long, narrow, approximately cylindrical, extending from base of praesoma through the trunk bulb and false neck into the cavity of the trunk; one with two giant nuclei and the other with one.

Genotype: Eocollis arcanus n. sp.

Eocollis arcanus new species
(Figs. 1–8)

With the characteristics of the genus *Eocollis* as enumerated above. Adults, so far as known, living in the intestine of fresh water fishes of the family CENTRARCHIDAE. Developmental stages and intermediate hosts unknown.

Largest females about 13 mm long of which the body bulb is 0.24 to 1.58 mm in length, the false neck occupies from 0.24 to 1.26 mm and the trunk bulb 0.24 to 1.58 mm in length; false neck 0.12 to 0.5 mm in diameter; maximum trunk diameter about 2 mm; bulb diameter 0.2 to 1.89 mm. Observed males somewhat smaller than females, to about 8 mm in length by 1.34 mm in maximum diameter. In some individuals, instead of a single, uniformly expanded, spheroidal trunk bulb the false neck may bear one or a few irregularly lobed expansions at the base of the proboscis; in a few the bulb is entirely lacking. Characteristically the narrow, approximately cylindrical false neck and enlarged trunk bulb closely resemble the attenuated cylindrical neck and neck bulb of members of the genus *Pomphorhynchus*. Proboscis globular or very short, cylindrical, from 0.076 to 0.117 mm long by 0.094 to 0.192 mm in diameter. The proboscis receptacle a very small closed sac, about 0.08 to 0.21 mm long by 0.05 to 0.067 mm in diameter, its wall consisting of a single muscular layer. A brain about 0.079 mm long is located at its posterior end. Retinacula not observed. In immature individuals lacking the trunk bulb, the receptacle seems to be considerably larger than in older and more fully developed specimens. Several relatively large muscles (Fig. 7) extend from the false neck region into the front end of the enlarged portion of the body cavity. These seem to be retractors of the neck although they could not be traced anteriorly through the false neck to the base of the praesoma. The heavy trunk bulb surrounding the base of the proboscis and the neck makes it impossible to

determine the length of the praesoma in all specimens. The cuticular ring which marks the boundary between trunk and praesoma is likewise obscured by the heavy folds of the trunk forming the trunk bulb. Proboscis armature consisting of three circles of six hooks each. Hooks very delicate, readily disturbed in removal from host intestine. Hooks of terminal circle about 0.047 to 0.059 mm long and about 0.006 mm in diameter at the bend where blade joins root; hooks of middle circle about 0.023 mm long; of basal circle about 0.012 mm long.

Lemnisci provided with a median canal (Fig. 7). The one with two giant nuclei but little longer than the one with a single nucleus, both reaching relatively far into the main body cavity; in the allotype male extending back to near the hind margin of the posterior testis. The two contiguous testes are very diverse in shape and size. In some individuals they are both much elongated with the posterior end greatly reduced in diameter. In the allotype male the anterior testis, which extends almost to the base of the false neck, is ellipsoidal, 0.75 mm long by 0.53 mm wide; the posterior testis is much elongated with the posterior end attenuated, 1.48 mm long by 0.46 mm wide. In some males the anterior testis reaches a length of 1.7 mm.

The large syncytial cement gland (Fig. 3, CG) which touches the hind margin of the posterior testis, contains eight giant nuclei. It has a length of 1.73 mm and maximum width of 0.44 mm in the allotype male, practically filling the body cavity. Cement reservoir pyriform, 0.35 mm long by 0.24 mm wide in a typical specimen.

Embryos within body cavity of gravid female 0.041 to 0.047 mm long by about 0.010 mm wide. Intermediate hosts and larvae unknown.

Definitive hosts.—In intestine of *Lepomis macrochirus* Raf. (the bluegill) of the Ohio River at Shawneetown, Illinois and *Pomoxis nigro-maculatus* (Le Seur) (the black crappie) of Horseshoe Lake near Cairo, Illinois.

Holotype female (VC 3828.4), allotype male (VC .3828.10) and a series of 18 paratypes in the collection of Harley J. Van Cleave, Urbana, Illinois.

Eocollis arcanus is so distinctively different from all other species of ACANTHOCEPHALA that the diagnostic features of the species as well as of the genus are best shown in a key which will serve for differentiating all representatives of the EOACANTHOCEPHALA known for this continent.

ARTIFICIAL KEY TO THE GENERA OF NEOACANTHOCEPHALA
KNOWN TO OCCUR IN NORTH AMERICA

1 (a) Proboscis shortly cylindrical or globular, hooks in three circles—2
  (b) Proboscis cylindrical, hooks in more than three circles—5
2 (a) Proboscis hooks six to a circle—3
  (b) Proboscis hooks more than 6 to a circle—4
3 (a) Praesoma joins trunk without any swollen transitional zone—*Neoechinorhynchus* Hamann in Stiles and Hassall, 1905
  (b) Anterior end of trunk modified to form a slender false neck and inflated trunk bulb at base of praesoma—*Eocollis* new genus
4 (a) Proboscis hooks twelve to each circle—*Gracilisentis* Van Cleave, 1919
  (b) Proboscis hooks eight to each circle—*Octospinifer* Van Cleave, 1919
5 (a) Proboscis at least 0.5 mm long, more than twice as long as broad. Longest hooks more than 0.050 mm long—*Tanaorhamphus* Ward, 1918
  (b) Proboscis small, about 0.15 mm long, nearly half as broad as it is long. Largest hooks under 0.020 mm long. *Atactorhynchus* Chandler, 1935

As knowledge of the acanthocephalan fauna of the New World unfolds, there is no aspect more striking than the extreme diversification of the representatives of the class EOACANTHOCEPHALA and particularly of the order NEOACANTHOCEPHALA. For the European fauna, nearly two centuries of continuous study have revealed only two species of the EOACANTHOCEPHALA both of which belong to the neoacanthocephalan genus *Neoechinorhynchus* (*N. agilis* and *N. rutili*), while the GYRACANTHOCEPHALA are entirely missing from the European continent.

Prior to 1913, all records of the occurrence of EOACANTHOCEPHALA in North America were erroneous accounts (Van Cleave, 1913) of one or the other of the

two European species or one of their recognized synonyms. Until 1913, consistent investigation of ACANTHOCEPHALA was confined to the Atlantic coast fauna with but little attention being directed to the parasites of fresh-water fishes from the interior of the continent. A distinctive fauna inhabiting chiefly the fishes of the Mississippi valley has gradually been revealed in the literature (Van Cleave, 1919). Comparisons of this with faunas of other regions of the earth have amply demonstrated the fact that diversification at both specific and generic levels has been unusually active in this region, resulting in the production of an extremely rich representation of the NEOACANTHOCEPHALA. To the writer it seems fruitful to draw comparisons between this diversification of the acanthocephalan fauna and speciation of the fishes which serve as definitive hosts for the NEOACANTHOCEPHALA.

In viewing the phylogeny of the EOACANTHOCEPHALA, it is the writer's belief that the GYRACANTHOCEPHALA are more generalized and more primitive than the NEO-ACANTHOCEPHALA. The former have had their most distinctive differentiation in the Old World. Of the four generally recognized genera of the GYRACANTHO-CEPHALA, three occur in the Orient (*Pallisentis, Neosentis* and *Acanthosentis*), one (*Quadrigyrus*) is known from South America only. GYRACANTHOCEPHALA seem to be entirely lacking from Europe and North America. Since all members of this order utilize fresh water fishes as definitive hosts the geographical distribution and limitations of the genera offer many interesting problems for speculation. It seems possible that at some earlier time the primitive GYRACANTHOCEPHALA may have been more widely dispersed on all continents with possible exception of Africa. Diversification of the original hosts may have been accompanied by parallel morphological and physiological changes in the parasites so that the primitive forms could no longer maintain themselves but gave way to the more specialized forms which marked the origin of the present day NEOACANTHOCEPHALA.

The NEOACANTHOCEPHALA have blossomed along with the diversification of the fresh-water fish fauna of the New World. The group has remained at a monotonous level in Europe with somewhat better showing in Asia and in South America but with extreme diversification in North America. *Neoechinorhynchus* is the only genus of this order established in the European countries. In Asia this same genus appears accompanied by two additional genera, *Hebesoma* and *Eosentis*. For South America there is no recent record of the occurrence of the genus *Neoechinorhynchus,* although Diesing's (1856) account of *Echinorhynchus variabilis* may readily be a *Neoechinorhynchus* which has not been recognized again in the past ninety years. *Pandosentis* is the only genus of NEOACANTHOCEPHALA which has been recorded for the continent of South America in recent years.

In fresh water fishes of North America, the NEOACANTHOCEPHALA have attained the extreme expression of their diversification with a total of 6 genera recognized. The genus *Neoechinorhynchus* is represented in North America by the holarctic species *N. rutili* encountered on this continent in the course of studies, the details of which are not yet ready for publication. It is further represented by a long series of forms which seem to have resulted from an almost explosive fragmentation into a large number of clearly marked species of which seven have already been described (*N. cylindratus, N. crassus, N. tenellus, N. emydis, N. australis, N. venustus* and *N. cristatus*) and at least three additional species are to be described in a forthcoming monographic treatment of the genus. The genus *Neoechinorhynchus* has attained

very wide geographical distribution but it also utilizes a very great number of genera of fishes as definitive hosts. However, some species like *N. crassus, N. venustus, N. cristatus, N. emydis* (peculiar in that it occurs exclusively in turtles) and *N. australis* are rather rigidly limited in their definitive hosts. The relationships of the hosts of a given species do not always depend solely upon taxonomic relationships for often agreement in habitat and in food habits is just as important in the determining infectibility as is agreement in taxonomic characters.

Evidences of evolution at the generic level are even more strikingly present than at the specific level for the NEOACANTHOCEPHALA of North America. Previously *Gracilisentis, Tanaorhamphus, Atactorhynchus* and *Octospinifer* have been widely accepted distinctive genera based upon sound morphological characters which include objective, qualitative distinctions in their characterizations. *Eocollis*, a sixth genus of NEOACANTHOCEPHALA for North America, is added to this list in the present contribution. Five of these six genera are distinctively North American and four are characteristically found in fresh water fishes although one species has been recorded from a catadromous eel while *Atactorhynchus* came from Galveston Bay (Chandler, 1935). The diversification of the acanthocephalan parasites of the fresh water fishes of North America is in a very real sense in keeping with known facts regarding diversification of the fish fauna of this continent. Jordan (1928: 376) has given a table in which he compares the speciation in families of fresh water fishes in Europe and North America. The four distinctive North American genera of NEOACANTHOCEPHALA reach maturity in families of fishes which are likewise distinctively North American and are wholly lacking in Europe.

Gracilisentis and *Tanaorhamphus* have *Dorosoma cepedianum* as their characteristic definitive host in a distribution from Illinois southward through the Mississippi valley. This genus of fish does not occur in Europe and according to most of the older classifications it belongs to a family (Dorosomidae) which is restricted to North America. There are only occasional and probably accidental records of the occurrence of either *Gracilisentis* or *Tanaorhamphus* in other species of fishes.

The genus *Octospinifer* is exclusively restricted to the Catostomidae over wide range of central and northeastern United States and in Canada as far west as Saskatchewan. Survey records from states south of Illinois have never recorded the presence of *Octospinifer* which seems to be distinctively a northern species. According to Jordan (1928) the Catostomidae which are so abundantly represented in North American fauna are lacking entirely in Europe. The genus *Eocollis*, the subject of the present paper, seems rigorously limited to southern Illinois where it occurs in Centrarchidae. This family of fishes is wholly missing in the European fauna.

Cyprinodon variegatus, in which Chandler (1935) found *Atactorhynchus verecundus*, belongs to the family Cyprinodontidae. Jordan, Evermann and Clark (1930) list 70 species of this family for North America while but three related species (Jordan, 1928) are found in Europe. The family is therefore distinctively North American and, like the other hosts of peculiarly American NEOACANTHOCEPHALA, the family shelters a characteristic acanthocephalan fauna. While many members of this family are fresh water inhabitants, Chandler found *Atactorhynchus* in fishes from the upper part of Galveston Bay with but sparse representation of the parasites in fishes from Galveston Island. This limited distribution suggests the pos-

sibility that the intermediate hosts may live in fresh or brackish water as has been so distinctive of the four other genera of NEOACANTHOCEPHALA limited to the North American continent.

On evidences such as these the writer has come to believe rather firmly in the hypothesis that speciation of fresh water fishes in North America has been paralleled by an integrated differentiation of species in the ACANTHOCEPHALA for which the viscera of the fish provide all of the essential features of the environment.

It has long been the writer's belief that the immediate progenitors of all ACANTHOCEPHALA were an ancestral stock intermediate between present day cestodes and present day acanthocephalans, existing in either the Cambrian or the Ordovician in a primitive ancestor of modern fishes or in a primitive arthropod. The regularity with which arthropods serve as the first intermediate host of modern acanthocephalans seems to favor the idea that these worms originally attained their full development in the arthropod host and added the vertebrate host as a secondary complication of the life history as fishes fed on the arthropod hosts. As the fishes underwent evolutionary changes, their parasites made parallel progress and differentiation. Then as each new group of the vertebrates appeared in the pageant of evolution it carried the acanthocephalan parasites which its more primitive ancestors has supported. Morphological and physiological changes which accompanied the evolution of the host altered the conditions of existence for the internal parasites, since the host is the external environment of an internal parasite. By the interaction of the genetic and the environmental factors, the acanthocephalans have undergone evolution parallel with the main currents of the evolution of the vertebrate series. Thus the most highly specialized groups of ACANTHOCEPHALA are now found in birds and mammals and many of the most generalized forms occur in fishes but the alignment of this phylogenetic series is not perfect. In any group of hosts and in any given locality various factors have often interrupted the natural course of development.

Glaciation in central Illinois, where *Gracilisentis* and *Tanaorhamphus* now occur, wiped out the fish fauna which lived there before the advent of the ice age. The streams that became established as the glaciers retreated were of necessity repopulated by the active migration of species from farther south beyond the controlling influence of the ice sheet. Except for immigration through the northwest land route between Eurasia and North America this must have been the chief source of fresh water fishes of the glaciated areas of Illinois and other glaciated regions of the northern states. The speciation which accompanied the diversification of the new fish fauna must have been accompanied by a parallel speciation of the acanthocephalan fauna. While few species of ACANTHOCEPHALA of fish are strictly specific in their host limitations, the peculiar forms such as *Gracilisentis, Tanaorhamphus, Atactorhynchus, Octospinifer* and *Eocollis* have apparently become so intimately attuned to given host species or host families that the only ready explanation of their dependence seems to be traceable to correlated evolution of parasites and their hosts.

Records of the world distribution of ACANTHOCEPHALA are not complete enough to permit of comprehensive mapping and of tracing of phylogenetic origins and migrations of host species such as Metcalf (1923) has been able to present for the opalinid parasites of Amphibia. However, in recent years the available information

on individual genera and species of ACANTHOCEPHALA has been expanding rapidly. The present paper is a contribution toward a partial understanding of the parallel evolution in the ACANTHOCEPHALA. These parallels take two courses: (a) agreement in fundamentally different structures in acanthocephalans that are widely separated in taxonomy and (b) correlation of evolution of the parasites with their definitive hosts.

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EXPLANATION OF FIGURES
Details in the anatomy of Eocollis arcanus n. sp.

All drawings were made by Katharine Hill Paul, scientific artist in the department of Zoology and Physiology in the University of Illinois, from stained permanent mounts.

PLATE I

FIGS. 1 to 3. General topography of entire worms. The scale between Figs 2 and 3 applies to this group, having the value of 0.5 mm.

FIG. 1. Immature female from Pomoxis nigro-maculatus. TB trunk bulb, FN false neck. Note the five giant nuclei in the dorsal (left) wall of the body and the single one in the ventral wall just back of the false neck.

FIG. 2. Very young female from Lepomis macrochirus, lacking trunk bulb. L1 lemniscus with a single giant nucleus, L2 lemniscus with two giant nuclei.

FIG. 3. Mature male from Pomoxis nigro-maculatus. VN subcuticular nucleus in ventral body wall. CG syncytial cement gland.

FIG. 4. Terminal view of a proboscis showing the apical circle of hooks. Scale accompanying this drawing, 0.1 mm long.
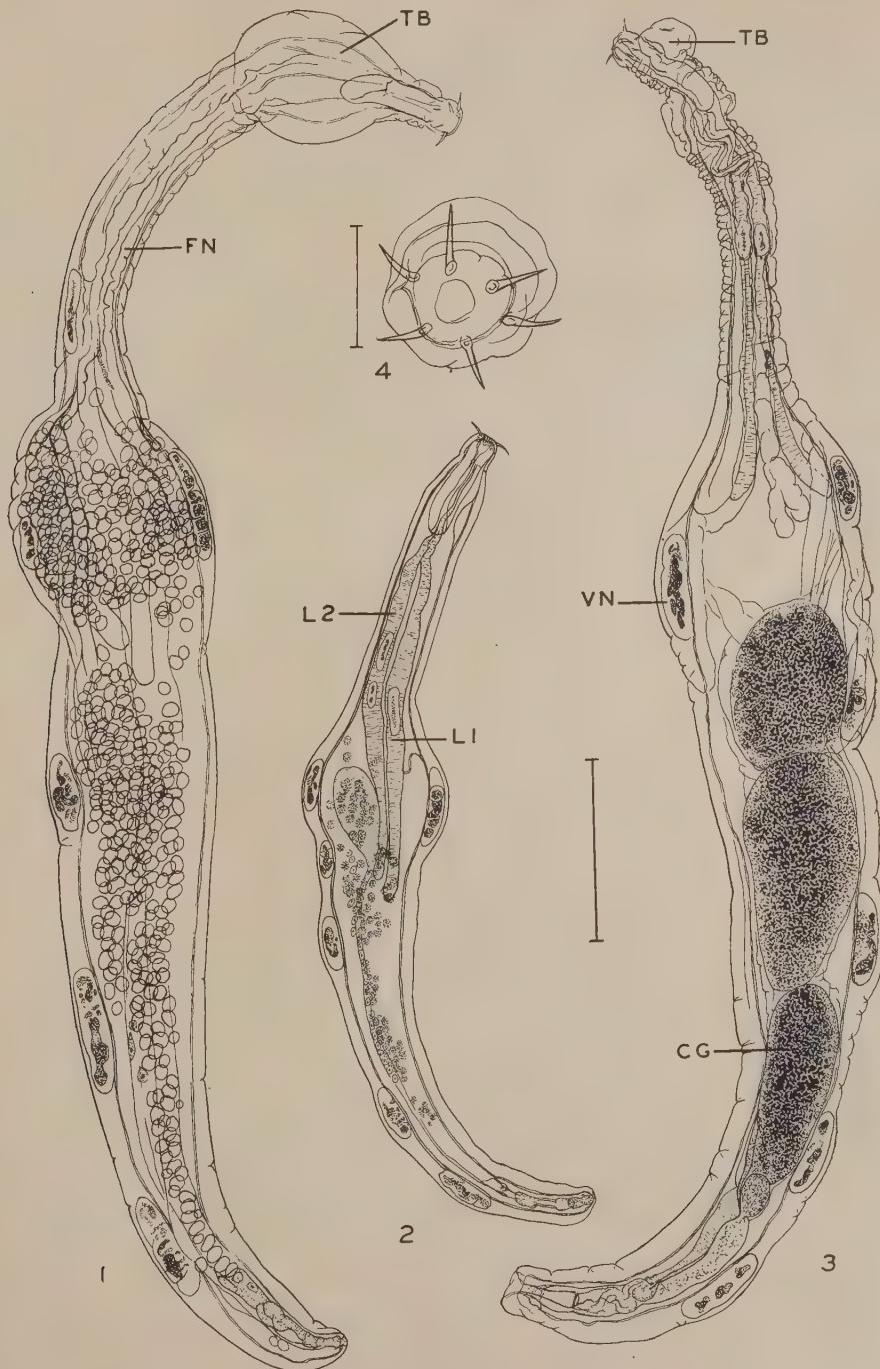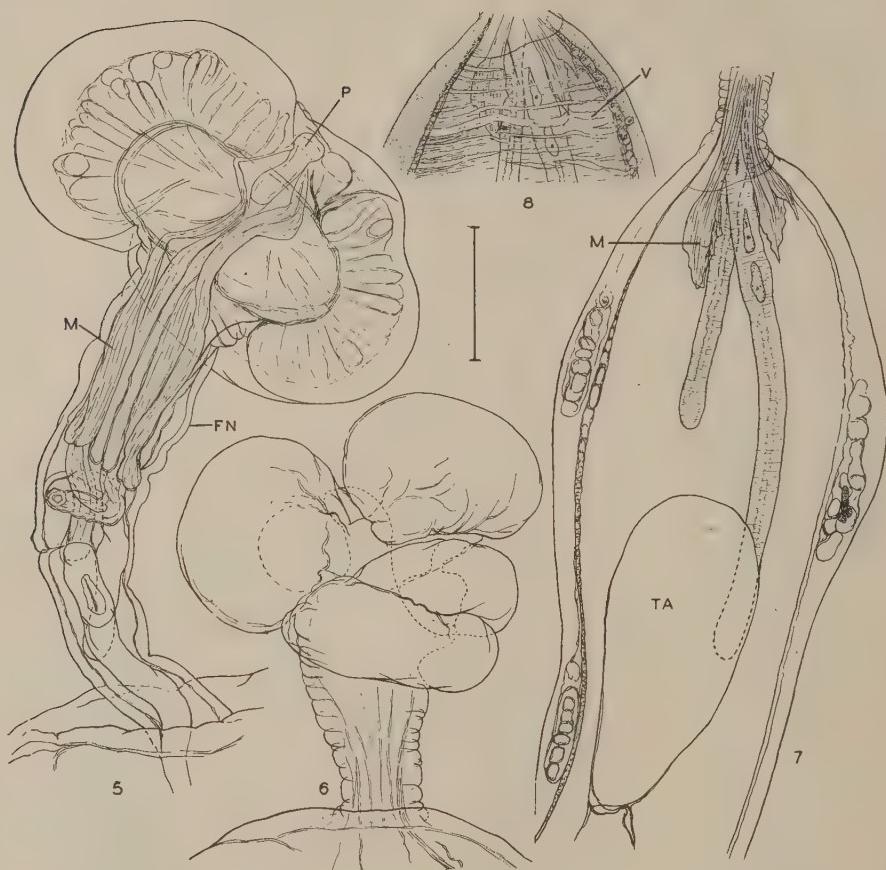
PLATE I

PLATE II

FIGS. 5 to 8. Details of structure of the trunk bulb, false neck and anterior region of the body proper. Scale has the value of 0.5 mm.

FIG. 5. Normal attachment organ and false neck of a female from *Lepomis macrochirus* showing characteristic hypodermal structure of trunk bulb. Proboscis (P) much obscured by surrounding walls of bulb. FN false neck. M special musculature for operating false neck and bulb.

FIG. 6. Attachment organ of a mature female from *Lepomis macrochirus* showing a cluster of excrescences instead of a well formed trunk bulb.

FIG. 7. Anterior extremity of body proper of a young male from *Lepomis macrochirus* in optical section showing long, cylindrical lemnisci, each with a median canal. The lemniscus with two nuclei extends well past the front edge of the anterior testis (TA). Special musculature for retracting neck at M. Large structures in body wall are hypodermal nuclei.

FIG. 8. Surface view of portion of same individual shown in Fig. 7, showing deflection of lacunar vessels (V) from their normal course due to localized tension at points of attachment of retractor muscles of false neck.

THE ACANTHOCEPHALAN GENUS *MEDIORHYNCHUS,* ITS HISTORY AND A REVIEW OF THE SPECIES OCCURRING IN THE UNITED STATES

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In their adult state members of the acanthocephalan genus *Mediorhynchus* occur exclusively in the intestines of land birds. Representatives of this genus have attained practically world-wide distribution but in attaining this wide geographical dispersal, speciation has often been achieved in rather restricted areas and by fairly definite limitation of the definitive hosts. The writer has in his possession collections from various parts of the earth. These contain several undescribed species, not treated in the present paper, which will be confined to the species known to occur in the United States, with mention of other forms only as they concern the American fauna or the history of the concept of the genus.

The migratory habits of the definitive bird hosts and the unsatisfactory nature of the early specific descriptions of the worms have imposed many temporary obstacles to a final analysis of the forms included within this genus. Indefiniteness of early description has in some instances led to an assumption of wide geographical distribution and host tolerance of a species where two or more species were actually involved. At the same time unwarranted belief in host specificity has led many workers to name as new, species which later were proved to be identical with others previously described. Although some of the features characteristic of the members of this genus were recognized by early investigators, the relationships between species now attributed to this genus were often grossly misinterpreted.

ORIGIN AND HISTORY OF THE GENERIC CONCEPT

Throughout the nineteenth century the species of this genus which were then known were invariably referred to the genus *Echinorhynchus.* Early in the following century workers on the Acanthocephala began to attempt to use the new classification which Hamann had proposed in 1892, when he made the first constructive contribution toward recognition of genera and families within the Acanthocephala. The only group which Hamann proposed at that time which seemed capable of including the forms now attributed to *Mediorhynchus* was the genus *Gigantorhynchus* within the family Gigantorhynchidae. Soon thereafter several investigators assigned species, which are now recognized as belonging to *Mediorhynchus,* to the genus *Gigantorhynchus.* This was particularly true of the large forms because many of the investigators of that day were taxonomists almost wholly unfamiliar with the morphological and cytological details on which Hamann based his pioneer studies in acanthocephalan taxonomy. Thus de Marval (1904: 582) gave recognition to a species which he considered as new under the name *Gigantorhynchus mirabilis* although he failed to observe that it was in close morphological agreement with another species which he listed in the same paper under the genus *Echinorhynchus* as *E. otidis.* Some investigators have overemphasized minor differences between *E. otidis* and other species closely agreeing with it in fundamental morphol-

ogy to the end that they have claimed that *E. otidis* represents a genus distinct from *Mediorhynchus*. As one result of the present study both *E. otidis* and *G. mirabilis* are recognized as belonging to the genus *Mediorhynchus,* as will be shown in a later part of this contribution.

The nomenclatorial history of the genus *Mediorhynchus* has been long and intricately involved. Skrjabin (1913) used the name *Gigantorhynchus* for a form which is clearly a member of the genus *Mediorhynchus,* when he described *Gigantorhynchus empodius* as a new species. As a natural generic group the concept of *Mediorhynchus* dates from 1914 but the name *Heteroplus* proposed by Kostylev (1914a) was a homonym and subsequently the synonymy became greatly confused. For forms that are as distinctive as those belonging to the genus *Mediorhynchus,* it is unexplainable how species could have been well known for more than 90 years before their common generic peculiarities were ever recognized. By a series of fortuitous circumstances that were obviously unrelated and apparently without any common background of causal relationship, the period from 1914 to 1917 witnessed the publication of results of three entirely independent programs of study that had been carried out in three continents; Europe, South America and North America. It is necessary to recall that this period marked an era of rather strict isolation of programs of pure science during and immediately following the first World War. The importance of considering these overlapping programs of research as due to coincidence, rather than regarding them as resulting from intentional or unintentional exchange of ideas, is particularly obvious because there was little opportunity for personal communications between the workers.

Nicholas Kostylev, a Russian scientist, in 1914 proposed the name *Heteroplus* for a generic concept based on *Echinorhynchus otidis* Miescher, 1841, as genotype. He recognized *Gigantorhynchus mirabilis* de Marval, 1904 as pertaining to the same genus and announced the belief that *E. taeniatus* von Linstow, 1901, is a direct synonym of the genotype. Kostylev's conclusion regarding the generic standing of these species was valid but unfortunately the name *Heteroplus* which he chose was preoccupied by its earlier use by Mulsant and Rey as name for a subgenus of beetles and hence as a homonym is unavailable for his generic concept in the ACANTHOCEPHALA. The date on which *Heteroplus* was applied in the COLEOPTERA is much confused in the literature but all references place it well ahead of Kostylev's use of the term. In the three leading nomenclators, the date for *Heteroplus* Mulsant and Rey is given as: 1858, 1868 and 1871.

Entirely unaware of the work by Kostylev on old world species, the present writer was engaged in a study of avian ACANTHOCEPHALA of North America when he encountered representatives of three previously undescribed and unmentioned species which he attributed to a new genus *Mediorhynchus* (Van Cleave, 1916). In spite of the attempt of some authors (for example, Meyer, 1932) to distinguish between this concept and the one which Kostylev named *Heteroplus,* the two concepts are identical. *Heteroplus* as applied to an acanthocephalan genus is the exact equivalent of *Mediorhynchus* and therefore must be regarded as a direct synonym of *Mediorhynchus*. The significance of the simultaneous programs of research on European and North American species becomes particularly noteworthy when it is recalled that no species compatible with this generic concept had ever been recognized previously for the North American fauna.

At about the same time, Lauro Travassos (1916), investigating the acantho-cephalan fauna of Brazil, independently gave recognition to this same concept. Travassos first applied the name *Empodius* as a generic designation to this concept in a publication whose exact date is highly controversial. In 1924 Travassos (page 365) definitely stated that the article in which the present writer (Van Cleave, 1916) proposed the name *Mediorhynchus* appeared a little while before the publication in which he (Travassos, 1916) proposed the name *Empodius*. In 1920 (page 9) Travassos had already acknowledged *Empodius* as a direct synonym of *Medio-rhynchus*. In the meantime the present writer (Van Cleave, 1918) added confusion to the problem of synonymy by attempting to distinguish between *Mediorhynchus* and some of the forms which had been attributed to *Empodius* and *Heteroplus*. This unfortunate attempt was based upon an unconfirmed error in observation but it was accepted by Travassos (1924) and was incorporated into the system of classification advanced by Meyer (1931) and adopted in his monograph (1932–1933).

In the meantime the nomenclatorial history of the concept became even more in-volved. In 1917, Travassos was obviously unaware of the fact that *Mediorhynchus* had been proposed as a generic name because in that year he made no reference to that name when he discussed the genus under his name *Empodius*. This omission does not indicate an oversight on the part of Travassos since the publication in which *Mediorhynchus* was proposed was issued in December, 1916 and because of war con-ditions probably did not reach Brazil until after Travassos had submitted his manu-script which was published in 1917. In that paper Travassos assumed that the two species which had formerly gone under the names *Echinorhynchus emberizae* Rud., 1819 and *E. micracanthus* Rud., 1819 are generically distinct from the concept of *Mediorhynchus* (= *Empodius*). He examined specimens which had been identified as *E. emberizae* and was unable to distinguish any thorns on the basal section of the proboscis, which he in error called the neck. On this presumed point of difference he proposed a new genus to which he applied the name *Micracanthorhynchus* and designated *E. emberizae* Rud., 1819 as its genotype. Apparently assuming that the thorns are likewise lacking from the basal part of the proboscis in *E. micracanthus* Rud., 1819 he named this as an additional species of *Micracanthorhynchus*. After an examination of many hundreds of stained permanent mounts of *Mediorhynchus,* the present writer is free to admit that in many individuals thorns may not be seen at all or are recognizable only with the greatest of difficulty. Often only the basal root of the thorn is recognizable on the proximal region of the proboscis and in many instances the small circular openings through which the thorns protrude are the only evidences that are available.

In 1924 the present writer recorded the results of an examination of the original material on which Rudolphi based his *Echinorhynchus micracanthus*. This was made possible by the generous cooperation of the Director of the Berlin Museum. The "types" had been preserved for more than a century yet they were in relatively good condition. While the thorns on the basal region of the proboscis were very small and inconspicuous they are present on that species. Here again independent discoveries were announced almost simultaneously after seven years of unhurried and unbiased consideration of the evidences uninfluenced by personal exchange of ideas. In 1924, Travassos stated that upon reexamination of specimens of *E. emberizae* he had verified the fact that hooks are likewise present on the basal region of the

proboscis of that species. Previously in a definition of the genus *Micracantho-rhynchus* and a description of *M. emberizae* (1917:60) Travassos had stated: "neck present, thornless, conical." When he corrected this observation in 1924 the sole basis for distinguishing between *Mediorhynchus* and *Micracanthorhynchus* was eliminated and the latter became, as Travassos admitted, a direct synonym of the former.

As previously mentioned, the unsettled world conditions accompanying and following the first World War were largely responsible for the nomenclatorial confusion here narrated. Exchange of publications between foreign countries was delayed and bibliographic services were seriously interrupted and impeded to the end that general availability of results of research was often long delayed. When the present writer first received a copy of Skrjabin's (1913) paper he was not then aware that *Heteroplus* as a homonym was not available. In 1918, he expressed the belief that *Heteroplus* might be regarded as distinct from *Mediorhynchus* on the erroneous assumption that the armature of the basal region of the proboscis followed a consistently different pattern in the two groups of species. Consequently (Van Cleave, 1918) *Mediorhynchus grandis* Van Cleave, 1916 was reassigned as *Heteroplus grandis* (Van Cleave, 1916). This assignment was based on an error in interpretation and as shown in a later work (Van Cleave, 1924:304) the proper designation for this species is *Mediorhynchus grandis*.

Travassos (1924:365) acknowledged, at least in principle, the attempt to revive a second generic concept, presumably distinct from *Mediorhynchus*. He therefore proposed that since the name *Heteroplus* was unavailable the revived concept should bear his name *Empodius*. This attempt at revival of a direct synonym as a generic name is in open violation of Article 36 of the International Rules of Zoological Nomenclature (1926:87) which states that "Rejected synonyms can be used again in case of the restoration of erroneously suppressed groups." Empodius was founded on *Gigantorhynchus empodius* of Skrjabin (1913) as genotype. Meyer (1932) and other authorities have without reservation assigned this species to the genus *Mediorhynchus*. Consequently since the species on which the genus *Empodius* was based is unquestionably included within the genus *Mediorhynchus* there is no ground for maintaining "erroneous suppression" of the name *Empodius* or the concept to which it was applied. The name *Empodius* cannot under the Rules of Nomenclature ever be revived as a valid generic name.

Southwell and Macfie (1925:162) refused to accept the proposal that *Empodius* is a direct synonym of *Mediorhynchus*. To quote their words: "In suggesting this synonymy, Van Cleave has apparently disregarded one of the characteristics of his genus *Mediorhynchus*, namely, that the wall of the proboscis receptacle is composed of a single muscular layer instead of two layers (a feature which is well shown in his figure accompanying his description of the type species *M. papillosus*) for in the genera *Heteroplus* and *Empodius* the proboscis-sheath has a double wall." This mistaken notion regarding the receptacle of some of the species which have been ascribed to *Empodius* or *Heteroplus* is due to the fact that the observers have misinterpreted drawings which have been presented for these species. Details of this confusion are presented in another paper (Van Cleave, in press). In brief summary, the individuals who have maintained that there is a double wall to the receptacle in forms ascribed to *Empodius, Heteroplus, Leiperacanthus* and some species of *Mediorhynchus* have been misled into interpreting a portion of the specialized musculature

for retraction of the neck and front part of the trunk as an integral part of the receptacle. There is no distinction of any sort between the receptacle in *Medio-rhynchus* and these forms which have been ascribed to other genera.

The untenable name *Heteroplus* continued to appear as a presumably valid name in literature as late as 1925 when Jean Baer described as *Heteroplus numidae* a species which he regarded as new and in the same paper reviewed the species of the genus using the generic name *Heteroplus* for all of the species of *Mediorhynchus*.

Anton Meyer, in his comprehensive monograph on the ACANTHOCEPHALA (1932–3), again attempted to revive the term *Empodius* as the generic name for certain species of *Mediorhynchus,* all different from the type which Travassos (1916) had designated, hence without nomenclatorial standing because all have no status outside the genus *Mediorhynchus*. Wholly aside from the question of infringement of the International Rules of Zoological Nomenclature the futility of the attempt to revive *Empodius* is revealed in Meyer's key for the separation of genera (1933: 532). The only features which Meyer found available to distinguish between his two artificial groups of species were: (a) texture of the egg membranes, (b) relative size of the body, and (c) the relative degree of pseudosegmentation. Two of these features (b & c) are wholly quantitative and the third (a), the texture of the membranes, is probably more of a quantitative than qualitative distinction. Relative differences are very generally recognized as of not more than specific value in taxonomy. The strong development of a regular pattern in the lacunar system in *Mediorhynchus* furnishes the morphological background on which pseudosegmentation is imposed. The relative development of the trunk musculature and relative development of the fibrous structures in the hypodermis determine whether the established regularity of the lacunar system shall be contained within a superficially smooth body surface or shall produce a series of regularly repeated annulations which are recognized as pseudosegmentation.

The species which Meyer segregated as representing his concept of *Empodius* are: *E. otidis* (Miescher), *E. taeniatus* (von Linstow), *E. numidae* (Baer) and *E. giganteus* (Meyer). A careful examination of the evidences available for these species fails to reveal a single point wherein they hold claim to generic assignment outside the genus *Mediorhynchus*. In his diagnosis of the genus *Empodius,* Meyer (1932: 178) stated that the proboscis is entirely cylindrical yet for two of his included species the shape is different from cylindrical. In *E. numidae* the proboscis has the same form of a truncated cone distinctive of most species of *Mediorhynchus* while in *E. giganteus* the proboscis consists of a globular portion attached to a conical base. Neither of these fits the characterization of the genus *Empodius* and this evidence shows conclusively how artificial are the grounds for the recognition of this group of species as a separate genus. Partial introversion of the tip of the proboscis seriously modifies the apparent shape of that organ. In forms which have the distal end of the proboscis normally in the shape of a truncated cone, partial introversion may transform this shape to globular or even cylindrical.

The genus *Leiperacanthus* which Bhalerao proposed in 1937 is likewise a synonym of the genus *Mediorhynchus,* as shown by the present writer in a paper dealing with ACANTHOCEPHALA as potential parasites of domestic poultry (Van Cleave, in press). This genus was founded on fundamental misconceptions of the structures encountered in a single specimen of a worm taken from the intestine of a domestic fowl in India.

Tubangui and Masilungan (1946) recorded the occurrence of this genus and the species on which it was founded in the Philippine Islands. While they extended the specific description considerably further than that given by Bhalerao they did not call attention to the errors in interpretation of some of the structures nor to the unsoundness of the genus. The paraproboscideal sacs which Bhalerao regarded as "altogether a new structure in the organization of the ACANTHOCEPHALA" have been observed in many species of *Mediorhynchus* and were figured by Skrjabin in his drawing of *M. empodius* (1913, fig. 13).

As summary of the foregoing section on the history of the generic concept of *Mediorhynchus* and presentation of the evidence for regarding other names as synonyms, the following synonymy has been established:

<div align="center">

Genus *Mediorhynchus* Van Cleave, 1916

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Synonyms: *Echinorhynchus,* in part
Gigantorhynchus, in part
Heteroplus Kostylev, 1914
Empodius Travassos, 1916
Micracanthorhynchus Travassos, 1917
Leiperacanthus Bhalerao, 1937

<div align="center">

THE GENOTYPE OF *Mediorhynchus*

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Mediorhynchus papillosus Van Cleave, 1916 was designated as the genotype of *Mediorhynchus* by Van Cleave, 1918. This subsequent designation was made necessary because no type of the genus was specified in 1916 when the genus was founded and named. Dr. C. W. Stiles in a personal letter called the attention of the writer to oversight in the 1916 paper, whereupon the present writer in a letter to Stiles indicated his intention of designating *M. papillosus* as genotype. In the Stiles and Hassall Index (1920) this letter is quoted as authority for the designation instead of the date of the published memorandum (1918).

<div align="center">

THE FAMILY ASSIGNMENT OF *Mediorhynchus*

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As early as 1917, Travassos assigned the concept of the genus *Mediorhynchus* (= *Empodius*) to the family GIGANTORHYNCHIDAE. This assignment has been accepted by almost all subsequent authorities, including Meyer (1931).

In the original characterization of the genus *Mediorhynchus* the present writer (Van Cleave, 1916) made a fundamental error in determining the relationships of this genus. He was greatly impressed by the fact that the proboscis receptacle is not inserted at the base of the proboscis but extends far forward within that organ. The only other acanthocephalans showing such a condition with which he was then familiar belonged to the genus *Centrorhynchus*. At the time this point of agreement seemed so important that the genus *Mediorhynchus* was at first erroneously placed in the new family CENTRORHYNCHIDAE (Van Cleave, 1916) which was constructed to include *Centrorhynchus* and *Mediorhynchus,* on the assumption that they were closely enough related to be included in the same family. This was an inexcusable error in judgment, since fundamental differences in morphology of the proboscis and of the receptacle were ignored in the union of these genera. In the following year (1917) Travassos assigned the concept of *Mediorhynchus* (= *Empodius*) to the family GIGANTORHYNCHIDAE.

In 1921 Travassos reduced the family Centrorhynchidae to subfamily rank, although the date on which this was done is misquoted by most subsequent writers. It seems to have been Travassos' practice to cite the date on which a paper was given on a scientific program rather than the date of publication. He prepared a bibliography of his own papers from 1913–1923 (Travassos, 1924?) in which he has given some evidence of this practice. In this list the paper which included the transfer of Centrorhynchidae to subfamily status is included with his list of works for 1919 although the actual reference to it in published form is cited as 1921.

The family Leiperacanthidae, which Bhalerao (1937) proposed to contain his presumably new genus *Leiperacanthus,* falls as a synonym to Gigantorhynchidae with the recognition of *Leiperacanthus* as a direct synonym of *Mediorhynchus.*

THE OCCURRENCE AND RECOGNITION OF THE SPECIES FOUND IN THE UNITED STATES

In 1916 when the writer established the genus *Mediorhynchus* and described three North American species belonging to it, he had available but a relatively small number of specimens for the entire study. Since that date many hundreds of specimens of this genus have been examined, including considerably more than 200 stained whole mounts from North American birds as well as representative collections from many other countries. In the original material on which descriptions of species were based, many individuals had the proboscis obscured by introversion or retraction. Specimens in this condition have relatively little value since number, size and relation of the hooks and their roots are not available. This factor led to the formulation of very incomplete and inaccurate descriptions of one of the species particularly. Most of the original material had been obtained in routine field examination of hosts. On the basis of small numbers of imperfect specimens a very incomplete concept of individual variability was gained for all the species originally described. In the present study many of the errors and omissions have been corrected. For a long time the writer interpreted the apparent irregularity in arrangement of the hooks on the proboscis as due to faulty killing or handling of the material but adequate material has shown that the proboscis hooks in this genus are not arranged in definite longitudinal rows. As Meyer (1931) has maintained for these forms, the hook arrangement is in the form of diagonal spirals. This departure from the arrangement in straight, parallel longitudinal rows renders enumeration extremely difficult. It is wholly impossible to follow spirals from one surface to the other, especially because the tips of the hooks are so frequently obscured by cuticular elevations of the body wall. Since number and arrangement of the hooks had been the chief characteristic on which species had been described it has been necessary in the present study to reevaluate the features on which species may be distinguished. On many individuals no traces of the thorns of the hooks on the anterior section of the proboscis are observable. At times of two specimens from the same host individual, carried through exactly the same staining and mounting technic, one will show thorns clearly while the other gives not the slightest evidence of thorns protruding from the proboscis surface and no indication that thorns have been broken from their root processes. Particularly in *M. papillosus* the papillose elevations (Figs. 10–12) around each proboscis hook often completely obscure the delicate tips of the thorns. As the eye follows the bend where the thorn joins the root it is often impossible to

distinguish in whole mounts between the hyaline thorn material and the curved out-line of the cuticular covering of the papilla. At least in the North American species the protruding points of the hooks are so indefinite and so variable that they have little value in specific diagnosis.

Through the generous cooperation of colleagues, a very extensive series of specimens has been assembled for the present study. This new material and a re-examination of the types have provided the basis for more precise specific discrimina-tions and have yielded much information regarding host relationships. The writer is especially indebted to Dr. Robert Rausch for numerous collections, preserved with great care, over a period of years and representing many host species from Ohio, Michigan, and Wisconsin. Among the many others who have submitted specimens that have made this study possible, are: Dr. Elizabeth M. Boyd, Dr. J. E. Ackert, Dr. Paul D. Harwood, Dr. F. R. Beaudette, Dr. John B. Loefer, Mr. Allen Mc-Intosh, Dr. D. R. Lincicome, and Dr. W. Henry Leigh.

Numerous collections from other countries have likewise been available for study and comparisons to make sure that the species from the United States were not representatives of species disseminated by migratory birds or by birds of wide geo-graphical distribution. The results of the investigation on specimens from other countries have not been incorporated into the present study except in so far as they have thrown light upon problems of generic characteristics and positive differentiation between species found in the United States and in other countries.

Within the United States there have been no records of the occurrence of *Mediorhynchus* west of a line connecting Lincoln, Nebraska; Manhattan, Kansas; and Houston, Texas. This line is not cited as a barrier to geographical distribution. It seems more probable that absence of records is due to failure to examine insect eating land birds or to publish the records of autopsies of these birds. Four species of *Mediorhynchus* are known from South America, chiefly through the works of Travassos. The writer has a single individual of an undetermined species from a western robin from Mexico furnished by Mr. Robert Traub of the Hoogstraal Ex-peditions and through the courtesy of Miss Marguerita Bravo Hollis of Mexico City has examined specimens of still another species from Mexico. It therefore seems evident that neither general ecological conditions nor absence of suitable definitive hosts can explain the lack of records of *Mediorhynchus* at least as far west as to the Rocky Mountains.

In many of the hosts which have been found to harbor *Mediorhynchus* the inci-dence of infection is extremely low. In many instances a single worm has been taken from one host individual while other birds of the same species taken at the same locality at the same time have been uninfected. The very low intensity of in-fection in the individual hosts is to be interpreted as giving evidence that reservoir intermediate hosts are lacking or at least play little rôle in pyramiding infections of the definitive host. This observation is in keeping with known feeding habits of the definitive bird hosts, most of which feed to some extent upon insects or other terrestrial arthropods which serve for the larval development of *Mediorhynchus*. Very few of the normal definitive hosts of *Mediorhynchus* would feed upon larger arthropods or vertebrates which might serve as reservoir hosts to concentrate the larval worms. In some of the species, especially *M. papillosus* and *M. robustus*, the low intensity of individual host infection seems to be compensated by appearance of

the worms in a variety of definitive hosts with similar food habits and living in comparable habitats. For a number of species of birds, *Mediorhynchus* has been found in a variety of host individuals but every female specimen of the worm has lacked shelled embryos even though the female may have attained approximately maximum size for the species. The significance of the presence of these sterile females is not fully understood. Their sterility may be seasonal or it might be due to the lack of males for fertilization of the eggs, or as yet another possibility their sterility might be a reflection of the fact that the physiological environment provided in the intestine of an abnormal host does not provide the conditions essential for the worms to reach functional sexual maturity. Possibilities of this sort are among the most eloquent of arguments against the meaningless extension of host lists by amassing individual instances of host species in which the parasite has been found without reference to the ability of the sheltering species to bring the parasite to functional maturity. Naturally there is no means of determining whether the sheltering vertebrate is a normal or accidental host until extended observations are available to determine if the worms ever reach maturity in the vertebrate under suspicion of being a normal link in the life cycle of the parasite. This topic has been much discussed by Lühe (1912: 277), by Meyer (1933: 313), and recently by Lundström (1942: 138). The latter refers to this abnormal relationship as host displacement. Many other terms have been suggested by various writers but all of them seem difficult of application because there is no direct test or criterion by which normal or anomalous rôle of the individual host is determinable. Only after the seasonal life history has been thoroughly investigated does it become possible to assay the significance of any presumed host species as normal or anomalous. Thus in order to avoid omitting names of vertebrates in which acanthocephalans have been found it seems desirable to include these in the lists of hosts until their status is finally determined. Consequently in the later part of this same paper some species of birds are cited as potential hosts even though the only records of the occurrence of the parasites are those in which immature individuals were encountered. Ultimately those species which are incapable of bringing the worms to full functional sexual maturity should be deleted from the list of hosts.

The scarcity of specimens and the peculiar distribution of the sexes in the final host is particularly well shown in the instance of *Mediorhynchus robustus*. In the material currently under investigation there has been no instance of male and female of this species found in the same host individual. This fact, coupled with the extreme sexual dimorphism to be mentioned later, imposed very severe difficulty in interpretation of the collections. In two different specimens of the towhee, males only of *M. robustus* were found, while in the fox sparrow, swamp sparrow, and brown thrasher females only have been found and these never more than two in a host individual. The low incidence of the occurrence of individuals in the hosts observed renders it necessary to have extremely great numbers of autopsies available before the status of any host may be determined.

In 1920 the writer (Van Cleave, 1920: 284) called attention to the pronounced sexual dimorphism found in *Mediorhynchus grandis*. In that instance the distinction between the two sexes is chiefly one of relative size of the trunk. In the course of the present study a much more pronounced dimorphism has been discovered in *M. robustus*. The proboscis in the two sexes of this species has hooks that are

distinctly different in size. · In fact the difference between males and females is so great that it is equivalent to the differences between distinct species in some other representatives of this same genus. The entire length of the roots of the largest hooks in males (Fig. 1) rarely exceeds 0.048 mm (range 0.038 to 0.048 mm), while in females (Fig. 2) the roots are but rarely as small as the largest on the male, ranging from 0.048 to 0.058 mm in length from posterior margin of the root to the bend where hook and root meet.

The complete life cycle has never been determined for any species of *Medio-rhynchus* found in the United States. All available evidence seems to indicate that the egg never hatches normally unless it is ingested by some suitable arthropod which serves as the first intermediate host. Manter (1928) published a preliminary memorandum on the artificial hatching of eggs of *Mediorhynchus*. He succeeded in artificially hatching *Mediorhynchus* eggs from the cowbird (*Molothrus ater*). In this experiment he followed the same technic which he had developed for artificially hatching eggs of *Macracanthorhynchus hirudinaceus*. Mature eggs taken from the body of a gravid female worm were put in water and were later allowed to dry. Upon rewetting the dried eggs, some of them broke open allowing the acanthor larva to escape. It should here be recalled that this larva is unlike the larva of many cestodes and trematodes which can actively seek out the larval host. Therefore while Manter's experiment provided a means of securing acanthors for study, it threw no light upon the normal life cycle of *Mediorhynchus*. Moore (1941 and 1942) determined experimentally that eggs of *M. grandis* will hatch normally in the digestive tract of certain grasshoppers (see host list) and will undergo full development through the series of acanthella stages in these insects. Moore has described infective juvenile stage from his experimentally fed grasshoppers; consequently it seems reasonably certain that birds receive their infection by feeding directly on the insect intermediate host without any reservoir host intervening in the life cycle. As mentioned elsewhere in this article the low incidence of individual infection in the definitive hosts is evidence that reservoir hosts are not available for pyramiding the infection in the final host.

The writer has spent many months in the analysis of some lots of specimens which seemed to show distinctive differences, possibly of specific significance. Individual variability, state of contraction of the proboscis, pronounced sexual dimorphism, and errors in the observations recorded in descriptions of the three species known from birds of the United States combined to increase the difficulty incident to specific determinations. Likewise differences in the methods of describing species has made comparisons between the forms found in the United States and elsewhere difficult and has always left the feeling that some species might be common to the two American continents through the agency of migratory birds. In the present study a thorough-going analysis of the descriptions of South American species and examination of two very small samples from Mexico have furnished evidence that in the new world there is no inter-continental dispersal of species in this genus through the agency of migratory birds. All three of the species occurring in the United States stand as morphologically distinct from species occurring in Mexico and South America.

In the following tabular key the analysis of features available for identification of the species found in birds of the United States is presented.

TABULAR KEY TO THE SPECIES OF *Mediorhynchus* KNOWN FROM LAND BIRDS
OF THE UNITED STATES

	M. grandis	M. papillosus	M. robustus
No. of approximately longitudinal rows of hooks on anterior segment of proboscis	18	18	20–24
No. of hooks in each diagonal row on anterior segment of proboscis	8–10	8–10	10–12
Length in microns of roots of hooks on anterior segment of proboscis	73–93	26–40	Male: 38–48 Female: 48–58
Dimensions in microns of embryos within gravid females	45–53 by 30	41–53 by 22–32	38 by 16
Length of females in mm	22–35	18–19	16–52
Length of males in mm	8	9	5.5 + –16

In the following section full specific descriptions will not be given. Only those points particularly distinctive of the species will be presented.

Mediorhynchus grandis Van Cleave, 1916
(Figs. 13–19; Text Fig. A)

Synonymy: *Heteroplus grandis* (Van Cleave, 1916) of Van Cleave, 1918
Heteroplus grandis (Van Cleave, 1916) of Baer, 1925
*Empodius grandis* (Van Cleave, 1916) of Travassos, 1924

Of the three species of *Mediorhynchus* known to occur in birds of the United States, *M. grandis* shows the sharpest limitation of its hosts. Grackles (*Quiscalus quiscula*) were cited as type host of this species while the eastern meadowlark (*Sturnella magna*) was mentioned as an additional host in the original description of the species. Later (Van Cleave, 1918: 29) the crow (*Corvus brachyrhynchos*) was added to the list. The grackles and meadowlarks seem to be the normal definitive hosts of this species while many other incidental hosts acquire the young worms by eating the arthropod hosts at the same foci of infection where the normal hosts acquire their worms. In several of these anomalous hosts fully matured eggs have never yet been found.

In the original characterization of *M. grandis* several errors in observation and interpretation were made, especially with regard to the number and arrangement of the proboscis hooks. In the type material of this species there was no individual with the proboscis fully extruded. Every one had the tip of the proboscis partially introverted or had the base of the terminal segment retracted and in some individuals these two conditions combined to wholly obscure features of the proboscis. Large numbers of fully extended individuals from the type host have been available in the present study and the results obtained from them have made modification of the original description necessary. Earlier in this paper attention has been directed to the fact that the proboscis hooks are not in alignment as perfect longitudinal and cross rows. The heavy wall of the proboscis obscures the hooks near the median plane of the proboscis on the dorsal and ventral surfaces as the specimen is viewed from its lateral aspect which is invariably uppermost in whole mounts of well-preserved material. Instead of the 12 longitudinal rows specified in the description of the species there are 16 to 18 approximately longitudinal rows when counted as members of a diagonal row in a zig-zag path around the circumference of the proboscis. One lateral surface shows 9 or 10 diagonal rows. Since the hook nearest the dorso-ventral sagittal plane can be seen from both lateral views, this enumeration checks

with the 16 or 18 diagonal rows for the entire front end of the proboscis. In those individuals which show the hooks as approximating longitudinal rows there are 4 or 5 hooks in each series. This corresponds to the 9 or 10 hooks which are visible in any complete diagonal row running from the base to the tip of the distal segment of the proboscis.

The roots of the proboscis hooks (Figs. 13 to 19) are by far the most distinctive feature for the recognition of *M. grandis* since they are much longer (0.073 to 0.093 mm) than in either of the two other species found in birds of the United States and they are considerably longer than measurements given by Travassos (1917, 1924) for all South American species except *M. pintoi* (0.078 mm). However, the embryos of *M. pintoi* (0.076 by 0.044 mm) are much larger than those of *M. grandis* (0.045 to 0.053 by 0.030 mm). On the basis of size and appearance of the roots of the largest hooks on the distal end of the proboscis, *M. grandis* is readily distinguishable from other species encountered in birds of the United States.

In most of the stained whole mounts of *M. grandis* the hooks and their roots were clearly differentiated. In many instances (Figs. 14, 15) a cuticular cowl is raised
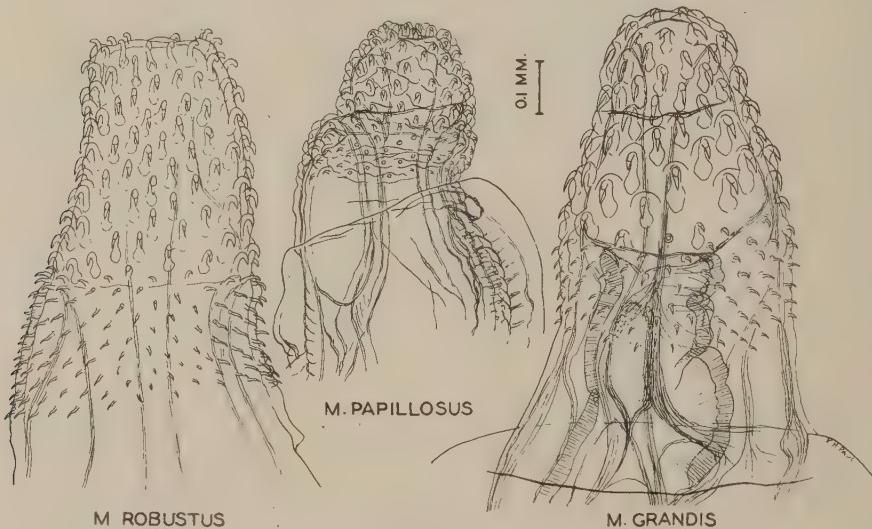
TEXT FIGURE A. A characteristic proboscis of each of the three species of *Mediorhynchus* occurring in birds of the United States. All drawn to the same scale to facilitate direct comparisons. The specimen of *M. robustus* is a female from a brown thrasher in Ohio (VC 3839). *M. papillosus* is a female from a vesper sparrow in Michigan (VC 2304.1). *M. grandis* is a female from a bronze grackle in Kansas (VC 676).

anterior to each large hook forming a very slight elevation homologous with the papillae found in *M. papillosus*. The cuticula likewise forms a distinct sheath or tubular sac through which the narrowed portion of the root passes without entirely filling the sheath (Figs. 15, 16). Similar sheaths around the roots of other species were not observed.

Definitive hosts of *M. grandis:* In the following lists, names of previously unpublished hosts and new localities are indicated by an asterisk (*) preceding the name of the host or of the state.

Quiscalus quiscula quiscula[1] (Linn.) (purple grackle): Maryland, *New Jersey

**Quiscalus quiscula aeneus*[1] (Ridgway) (bronze grackle): *Kansas, *Illinois, *Ohio, *Kentucky

Corvus brachyrhynchos Brehm (crow): Maryland, *Ohio

Sturnella magna (Linn.) (eastern meadowlark): North Carolina, *Ohio, *Illinois

**Agelaius phoenicius* (Linn.) (red-winged blackbird): *Ohio

**Euphagus carolinus* (Müller) (rusty blackbird): *Illinois

Arthropod hosts, experimentally determined by Moore (1941) in Texas:

Chortophaga viridifasciata australior

Orphuella pelidna

Arphia luteola

<div align="center">

Mediorhynchus papillosus Van Cleave, 1916
(Figs. 10–12; Text Fig. A)

</div>

Mediorhynchus papillosus was designated as type of the genus by Van Cleave, 1918. This subsequent designation was made necessary because no genotype was named at the time the genus was founded. All later studies have seemed to indicate that *M. grandis* might have been a more satisfactory choice as type because it is the most characteristic species found in the United States and all of the details of its anatomy are readily discernible in specimens which occur in relative abundance in commonly available species of definitive host.

In the original characterization of *M. papillosus* the presence of papillose elevations (Fig. 10) over the entire surface of the proboscis was acknowledged as the diagnostic feature suggesting the specific name. Similar elevations occur in varying degrees in other species of *Mediorhynchus*, even in the large *M. otidis* of Europe. The presence of these papillae to some extent obscures the hooks but since each prominence bears a single hook the pattern of hook arrangement is readily available even when some of the individual hooks are not distinct.

Hooks on the distal segment of the proboscis were, in the original description of the species, recorded as comprising 18 longitudinal rows of 4 to 6 hooks each. Here the same difficulty of enumeration was encountered as mentioned under *M. grandis*, due to the diagonal arrangement of the rows. Subsequent observations have verified the 18 rows but they are in diagonal series with 8 to 10 in each diagonal row. This is in accord with the drawing of a profile of the proboscis of the holotype male (Van Cleave, 1916, fig. 6) which shows 9 hooks. It is therefore clear that a diagonal row was figured rather than a single longitudinal series as mentioned in the description of the figure.

The shape of the roots of the hooks on the terminal segment of the proboscis (Fig. 11) is different in *M. papillosus* from that of the roots in any other species found in the United States. Each root consists of a narrow neck-like portion which bears the thorn and this posteriorly widens suddenly to form a circular basal disc. The over-all length of the larger roots in this species ranges from 0.026 to 0.040 mm, being thus the shortest roots found in any species occurring in the United States with the exception of some males of *M. robustus*. The smaller roots of this last

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[1] From data supplied by collectors, the varieties of the grackle are not always distinguished.

named species fall within the size range of the roots of *M. papillosus*. However this fact cannot lead to the confusion of individuals of these two species. The hooks of *M. robustus* are much more prominent and more abundant than those of *M. papillosus*. On the former they are definitely crowded (Figs. 1, 2) and lack the papillae distinctive of *M. papillosus* (Fig. 10).

In the course of the present study considerable difficulty has been encountered in the interpretation of the specific limits of *M. papillosus*. Many individuals have the proboscis partially introverted and retracted thereby masking the natural shape. This was particularly true of a short series of specimens from the song sparrow in which the shape of the proboscis seemed at first to be distinctive enough to warrant recognition of a distinct species. However, complete agreement in most other details led to the decision that the forms from the song sparrow are to be recognized as *M. papillosus*.

Leigh (1940) identified as *M. papillosus* two immature male individuals of *Mediorhynchus* from the prairie chicken in Illinois. The writer has reexamined both of these specimens which are in rather poor state of preservation and show the hooks and their roots in very unsatisfactory manner. It seems reasonably certain that Leigh was warranted in his specific identification although the alternative remains that these specimens might represent an undescribed species. Their physical condition and their immaturity render any attempt at more exact identification unprofitable.

In all instances that have come to light, every case of infection by *M. papillosus* has been extremely light. This low incidence of infection is in keeping with the relatively large size of the worms, especially the females, and the small size of the definitive hosts. Since so many of the hosts listed below are insect-eating birds, this fact with the low incidence of infection, supports the belief that the transmission to the definitive bird host is directly from arthropod host without intervention of any reservoir host.

M. papillosus was first described from the intestine of the eastern wood pewee, taken in Maryland by Albert Hassall in 1892. A single male of the same species was collected by Hassall from a sora rail likewise taken in Maryland. In Van Cleave, 1920 the locality of both of these collections was stated as unknown. Records in Stiles and Hassall, 1894 (page 352) give the locality for both as "Maryland."

Definitive hosts of *Mediorhynchus papillosus*:

Myiochanes virens (Linn.) (eastern wood pewee) : Maryland
Porzana carolina (Linn.) (sora rail) : Maryland
Tympanuchus cupido americana (Reichenbach) (greater prairie chicken) : Illinois
Sayornis phoebe (Latham) (eastern phoebe) : *Wisconsin
Pooecetes gramineus gramineus (Gmelin) (vesper sparrow) : *Michigan
Melospiza melodia melodia (Wilson) (song sparrow) : *Ohio

Arthropod hosts : wholly unknown.

<div align="center">

Mediorhynchus robustus Van Cleave, 1916
(Figs. 1–9; Text Fig. A)

</div>

M. robustus was originally described from one male and a single female both taken from the intestine of a yellow-breasted chat at Washington, D. C., by Albert Hassall. Since 1916, when the species was described, there has been no new informa-

tion on it in the literature. On the basis of the original specimens the writer was not particularly impressed by any size difference between the sexes. When material for the present study of *Mediorhynchus* was being assembled there were found several male individuals which seemed in size and shape of the body and in size, number and arrangement of the proboscis hooks to agree with the original concept of *M. robustus*. A total of 6 males were found in towhees, redwing blackbird and flicker but no female of *Mediorhynchus* was ever found in any of these host individuals bearing males of *M. robustus*. From the same general localities a few females of a *Mediorhynchus* were taken from swamp sparrow, fox sparrow, and brown thrasher but no males were associated in any instance. By strange circumstance no male has ever been found in the species of birds carrying the female worms and conversely no female has ever been taken from the species of birds in which the males were found. Rarely has there been more than a single individual of either sex in the infected bird host. The males and females of these two lots of specimens were different enough in size and shape of body and in size of the roots on the proboscis hooks that for some time they were suspected of representing two distinct species. As more material from the same localities became available, especially through the kind cooperation of Dr. Robert Rausch, the absurdity of the apparent segregation of males of "species A" in one series of hosts and only females of "species B" in another series became obvious. When similarities rather than differences between individuals of the two segregated sexes were looked for it began to be apparent that an instance of marked sexual dimorphism was involved, although this offered no explanation for the segregation. The available data are too fragmentary to permit of drawing any final conclusions but some possible interpretations will be mentioned. It is possible that the male may spend a shorter time than the female in the intestine of the final host. This statement is borne out by the fact that some females, found alone, were gravid indicating that a male must have been present at some earlier date and was expelled after fertilization had been accomplished. There is no doubt but that the relatively large size of the worms and small size of the host may be factors in the low incidence of infection. The method for maintaining this low incidence might be due to the presence of one or a few worms inducing some mechanism of immunity or resistance to reinfection in the already infected bird. Lack of massive infections in the definitive host would result in a thin spreading of the viable eggs passed with the feces of the host and this in turn would reduce the opportunity of eggs being ingested by a suitable arthropod since it seems very probable that this ingestion is accidental and incidental to the arthropod's feeding on vegetation contaminated with the feces from an infected bird. There thus seems to be a fine balance in feeding habits of the arthropod host and of the bird host contributing toward the establishment of light infections keyed to the size of the intestine of the definitive host.

By far the most distinctive feature available for recognition of *M. robustus* is the apparent crowding (Figs. 1 and 2) of the large hooks on the anterior region of the proboscis. The bases of the roots of these hooks (Fig. 8) are of approximately the same shape as in *M. grandis* but they are always shorter and are relatively narrower in the expanded proximal portion. This contrast is particularly evident when the proboscides of males are compared. The large roots of males of *M. robustus* measure from 0.038 to 0.048 mm, of females 0.048 to 0.058 mm in length; while those of *M. grandis* are 0.073 to 0.093 mm in length, without pronounced difference

between the sexes.   The embryos of *M. robustus* are the smallest for any observed in the three species considered, being especially narrower than in either of the other species.   There is some evidence that this may not be a valid criterion of difference since Travassos (1924) has shown that in species of *Mediorhynchus* which he studied the fixation and treatment of embryos resulted in conspicuous difference in size.

Definitive hosts of *Mediorhynchus robustus:*

> *Icteria virens virens* (Linn.) (yellow-breasted chat) : District of Columbia
>
> **Piplio erythrophthalmus erythrophthalmus* (Linn.) (towhee) : *Wisconsin
>
> **Agelaius phoeniceus phoeniceus* (Linn.) (red-winged blackbird) : *Ohio
>
> **Passerella illiaca illiaca* (Merrem) (fox sparrow) : *Wisconsin
>
> **Melospiza georgiana* (Latham) (swamp sparrow) : *Ohio
>
> **Turdus migratorius migratorius* Linn. (eastern robin) : *Ohio
>
> *Toxostoma rufum (Linn.) (brown thrasher) : *Ohio
>
> **Colaptes auratus luteus* Bangs (flicker) : *Illinois
>
> **Sturnus vulgaris vulgaris* Linn. (starling) : *New Jersey, *Indiana, *New York

Arthropod hosts : wholly unknown.

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Taxonomic characters for the recognition of the species of the genus *Mediorhynchus* known to occur in birds of the United States. Katharine H. Paul, scientific artist in the department of Zoology and Physiology of the University of Illinois, prepared the drawings from stained permanent mounts.

All six drawings of entire proboscides are to the same scale, indicated by the reference line to the right of Fig. 2, which has the value of 0.1 mm. All figures illustrating details of hooks and their arrangement are of uniform magnification and for them the 0.1 mm line between Figs. 3 and 5 applies. The numerals following VC in parentheses refer to slides in the collection of the writer.

FIGS. 1 to 9. Proboscis and details of hooks of *Mediorhynchus robustus* Van Cleave, 1916.

1. Fully extended proboscis of a male from a robin taken in Ohio (VC 3802).

2. Proboscis of a male taken from a red-winged blackbird taken in Ohio (VC 3806).

3. A series of adjacent hooks showing form, size and arrangement in the zone of transition between anterior and posterior segments of the proboscis. From lateral surface of female specimen from a fox sparrow taken in Wisconsin (VC 3940.1).

4. Two hooks from near base of anterior segment of proboscis in full profile view. Same individual as for Fig. 3.

5. A group of hooks from basal segment of the proboscis, in full profile. Same individual as in Figs. 3 and 4.

6. Proboscis of female from a swamp sparrow taken in Ohio. Note that the proboscis is slightly infolded at boundary between anterior and posterior segments (VC 3843).

7. Profile of hooks from near base of anterior segment of the same proboscis as that shown in Fig. 6.

8. Full front view of hooks from near base of anterior segment of the proboscis of the same individual as shown in Fig. 6, showing relative size and spacing of adjacent hooks.

9. Profile of hooks on basal segment of same proboscis as shown in Fig. 6.

FIGS. 10 to 12. Proboscis and details of hooks and their arrangement characteristic for *Mediorhynchus papillosus* Van Cleave, 1916.

10. Proboscis of female from vesper sparrow of Michigan, showing especially the characteristic papillose nature of the proboscis wall (VC 2304.1).

11. A cluster of hooks from near base of lateral surface of proboscis of a female, showing distinctive cuticular foldings and short, broad roots to the hooks (VC 2304.3).

12. Profile showing arrangement of thorns from basal segment of the proboscis, each in its own cuticular elevation. From same individual as Fig. 11 (VC 2304.3).

FIGS. 13 to 19. Characteristic proboscides and details of form and arrangement of the hooks and their roots in *Mediorhynchus grandis* Van Cleave, 1916.

13. Fully extended proboscis of a female from a bronze grackle taken in Illinois (VC 3880).

14. Three adjacent hooks from lateral surface of anterior segment of proboscis, showing characteristic shapes of hooks and their roots and the cuticular sheath through which the hooks extend. From same proboscis as shown in Fig. 13.

15. Profile view of a cluster of three hooks from near base of anterior segment of the proboscis shown in Fig. 13.

16. Profile of top margin of one side of proboscis shown in Fig. 13.

17. Fully extended proboscis of a male from a meadowlark taken in Illinois (VC 2494.4).

18. Profile of hooks at base of anterior segment of proboscis of a male taken from a meadowlark in Ohio, showing hooks and their roots in characteristic arrangement (VC 3837).

19. Five simple thorn-like hooks from basal segment of same proboscis as those in Fig. 18.

K H PAUL

ANDRYA SCIURI N. SP., A CESTODE FROM THE NORTHERN FLYING SQUIRREL

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Among helminths taken from four northern flying squirrels (*Glaucomys sabrinus macrotis* Mearns) were two cestodes belonging to the genus *Andrya*. These represent an undescribed species.

The infected flying squirrels were collected in or near tamarack bogs during February, 1947, near Millston, Jackson County, Wisconsin.

Andrya sciuri n. sp.

Diagnosis: Length of strobila about 17 cm; maximum width 2 mm. Mature segments much broader than long, with a gradual increase in length toward posterior end of strobila, where maximum width is also attained. Ventral excretory canal averages 30 μ in diameter; dorsal and transverse canals much smaller. Scolex 380 μ in diameter; suckers weakly-muscled and inconspicuous, measuring about 150 μ in diameter. Slight segmentation evident immediately posterior to scolex. Genital *Anlagen* appear typically early. Genital pores nearly all dextral; situated in posterior third of segment. Genital ducts dorsal to excretory canals. Cirrus sac muscular; averages 200 μ long by 85 μ wide. Medial end of cirrus sac does not reach excretory canals. Internal and external seminal vesicles well developed; latter reaches size nearly equal to that of cirrus sac. Internal seminal vesicle enlarges toward posterior end of strobila; appears to persist throughout. Cirrus unarmed. Testes 100 to 110 in number; from 40 to 50 μ in diameter; not all in same plane. Testes pass lateral margins of excretory canals both porally and aporally; greater number of testes concentrated on aporal side of segment. Prostate gland absent. Vagina posterior, and at times partly ventral, to cirrus sac; large seminal receptacle appears medial to end of cirrus sac. Seminal receptacle reaches maximum size of about 420 μ long by 180 μ wide, after which it decreases in size, and gradually disappears. Ovary, with vitelline gland, situated at middle of segment. Development of uterus typical for genus. Terminal segments completely filled with eggs; latter measure from 52 to 56 μ. Embryo about 33 μ long. Pyriform apparatus strongly developed.

Host: Glaucomys sabrinus macrotis Mearns (Northern Flying Squirrel).
Locality: Millston, Jackson County, Wisconsin.
Habitat: Small intestine.
Type: Two slides containing an entire specimen have been deposited in the collection of the U. S. National Museum.

DISCUSSION

Of the species of cestodes previously attributed to the genus *Andrya*, at least eight appear to be valid. *Andrya sciuri* is readily differentiated from these by the number and distribution of the testes. *Andrya cuniculi* (Blanchard, 1891), *A. neotomae* Voge, 1946, and *A. monodi* Joyeux and Baer, 1930, have the testes confined to the area between the longitudinal excretory canals. *A. cuniculi* also reaches a large size not attained by other members of the genus. In addition to the difference in arrangement, the testes are fewer (60 to 74) in *A. neotomae* and (15) in *A. monodi*. *Andrya rhopalocephala* (Riehm, 1881), *A. cuniculi*, and *A. primordialis* Douthitt, 1915, all possess well-developed prostate glands; this structure is absent in *A. sciuri*. Moreover, *A. rhopalocephala* and *A. primordialis* have fewer

testes (75 to 80, and 20 to 40, respectively), and their arrangement differs from that in *A. sciuri.* *Andrya macrocephala* Douthitt, 1915, is easily differentiated from the present species by the size of the ventral excretory canals. *Andrya macrocephala* has fewer testes (43 to 57), and these overlap the excretory canals only on the aporal side; the scolex is also more strongly developed. *Andrya africana* Baer, 1933,

300u

1

2   300u
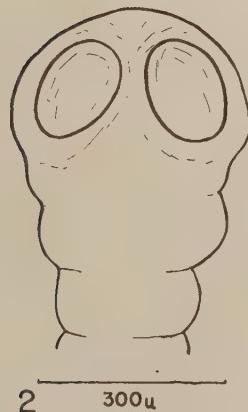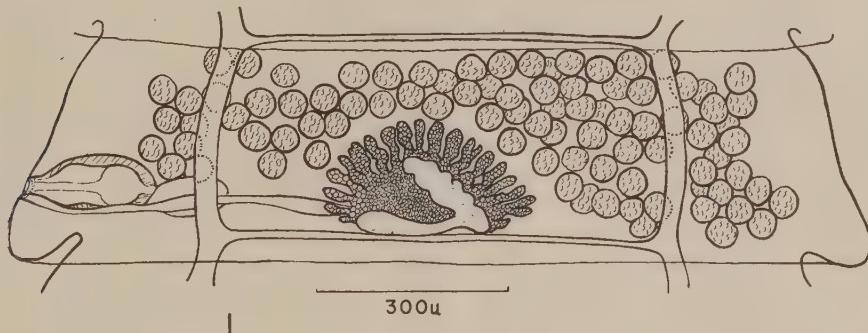
FIG. 1. A mature segment, ventral view, from *Andrya sciuri.*
FIG. 2. Scolex of *Andrya sciuri.*

has fewer testes (26 to 30) which nearly surround the ovary and vitelline gland, and the genital pores are unilateral. According to Baer (1927), Joyeux recognized a sub-species of *A. primordialis,* designated as *A. primordialis* var. *gundii.* This sub-species differs from *A. primordialis* only in number of testes (70 to 80) and in geographical location. The paper describing *A. caucasica* Kirschenblatt, 1938, was not available in the United States. The writer was advised by Dr. Charles Elton that this paper also is not available in the library of the Bureau of Animal Population, at Oxford. *Andrya caucasica* cannot therefore be considered in this discussion.

Two new host records have been added by the writer for cestodes of this genus: *A. macrocephala* was found to be a common parasite of the eastern meadow vole (*Microtus p. pennsylvanicus* Ord) in both Ohio and Michigan, and a single cestode of *Andrya* sp. was taken from a muskrat (*Ondatra z. zibethica* L.) in Ohio. It is hoped that additional specimens can be obtained from the latter host, since further study is desirable in order to establish specific identity.

A key has been prepared for cestodes of the genus *Andrya* found in North American hosts. Four species have been recorded, all of which are easily differentiated.

KEY TO THE NORTH AMERICAN SPECIES OF *Andrya*

1. Testes confined to the area between the longitudinal excretory canals 2
   Testes overlapping the longitudinal excretory canals 3
2. Testes 60 to 74 in number; prostate gland absent; ventral excretory canals not
   enlarged .... *A. neotomae*
3. Testes overlapping longitudinal excretory canals on both sides 4
   Testes overlapping longitudinal excretory canals on aporal side only 5
4. Testes 100 to 110 in number; prostate gland absent; ventral excretory canals not
   enlarged .... *A. sciuri*
5. Testes 20 to 40 in number; prostate gland present; ventral excretory canals not
   enlarged .... *A. primordialis*
   Testes 43 to 57 in number; prostate gland absent; ventral excretory canals
   greatly enlarged .... *A. macrocephala*

The writer wishes to take this opportunity to express indebtedness to Dr. E. W. Price for information from the files of the Bureau of Animal Industry, and to Messrs. Paul F. Springer and Alfred G. Etter, Department of Wildlife Management, University of Wisconsin, for aid in the collection of the animals from which this cestode was described.

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LÜHE'S *"DIPHYLLOBOTHRIUM"* (CESTODA)

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The generic term *Diphyllobothrium* was coined by Cobbold (1858) for five tapeworms taken from a porpoise, *Delphinus phocaena,* in Scottish waters, and was eventually adopted by Lühe (1910) in place of his previously (1899) suggested generic term *Dibothriocephalus* for a group of forms centering around the Linnaean species *latus.* It is a cumbersome group of about 70 species—many of them of dubious validity—and comprises forms from toothed whales, seals, sea-lions, carnivorous land mammals and fish-eating birds. Several species have been recorded from humans and one even from a snake. It has always been an unsatisfactory genus to define and analyze, and particularly difficult to evaluate have been the forms from seals and sea-lions that have been recorded by numerous writers.

An examination by the writers of a range of such material from seals in Canadian Atlantic waters and from sea-lions in Alaskan waters uncovered the following types.

1. Short muscular forms with the scolex short, thick, heart-shaped in marginal outline and up to 500 μ wide, triangular or oval in surficial outline (bothrial view) and up to 300 μ wide. Bothria deep, gaping anteriorly, narrower posteriorly, so as to appear keyhole-like, and extending the full length of the scolex, with margins prominent. No glandular tissue seen within the scolex parenchyma. Neck short, hidden usually by the overhang of the scolex. Body short, readily fragmenting and consequently difficult to estimate in total length; up to 6.5 mm wide and 1.25 mm thick. Segments extremely short and craspedote. Strobilar margins markedly serrated. Cirro-vaginal pore of the relaxed segment at the junction of the first and second thirds of the ventral surface, in the contracted segment being nearer to the anterior border and hidden usually by the overhang of the preceding segment. Parenchymal muscle zone well developed, appearing in a cross section as a conspicuous, deeply staining, continuous band, as thick dorso-ventrally as the enclosed medulla. Two sets of genitalia per mature segment. Testes large, reaching from floor to ceiling of medulla, appearing in a cross section of the segment as a single layer of 15–20 follicles between each margin and the nearest uterus, and as a double or triple layer of 10–12 follicles between the two uteri. Each cirrus pouch one-third as long as the medulla is thick, with a dorsal, external seminal vesicle, half its length. Yolk glands forming an almost uninterrupted band in the cortex, thinning, or very occasionally lacking, opposite the distended uteri. Each uterus with 3–4 loops on each side, closely appressed and parallel, the terminal loop distended and level with the cirrus pouch. Intra-uterine eggs averaging 50 by 40 μ, with rounded ends. Adults in seals (*Phoca groenlandica, Erignathus barbatus*) in Labrador waters.

This type recalled the descriptions of *Diplogonoporus fasciatus* (Krabbe, 1865).

2. Short muscular forms with the scolex also short and thick, heart-shaped or broadly oval or rectangular and 400–1000 μ wide in marginal outline, triangular or lanceolate and 800–1400 μ wide in surficial outline (bothrial view). Bothria deep, slit-like or keyhole-like, gaping anteriorly, narrowing posteriorly. No glandu-

lar tissue seen within the scolex parenchyma. Neck short, commonly hidden by the scolex overhang. Segments extremely short and craspedote, commonly widening rapidly from the neck backwards so as to give the body, in surficial outline, an acutely triangular shape. General internal features as in Type 1, but only one set of genitalia per segment present, and the ring of yolk glands interrupted in the mid-dorsal and mid-ventral lines by gaps one sixth the segment width. Testes spheroidal, 80μ in diameter, almost filling the medulla from floor to ceiling; appearing in cross sections of the segment as two layers or as one layer, according to the degree of segment contraction. Ovary as two flattened lobes. Uterus with 3–4 closely appressed loops one each side, sometimes, through secondary forking, giving the appearance of as many as seven loops on each side. Adults in seals in Labrador waters.

This type agreed with the descriptions that have been published of *Diphyllobothrium lanceolatum* (*vide:* Zschokke, 1903; Cholodkovsky, 1914; Stunkard and Schoenborn, 1936; Lyster, 1940).

3. Forms with the scolex relatively large, up to 2 mm in length, heart-shaped and up to 1.5 mm wide in marginal outline, oval and up to 1.25 mm wide in surficial outline (bothrial view). Bothria lanceolate or keyhole-like. No glandular tissue seen within the scolex parenchyma. Neck short, sometimes hidden but commonly visible. Segments varying from quadrate to linear. Cirro-vaginal pore of the relaxed segment slightly antero-central, of the contracted segment at the junction of the first and second thirds of the ventral surface. Vaginal and uterine pores slit-like. Ventral body surface with four equidistant, shallow, longitudinal furrows; corresponding dorsal furrows less apparent. Relative thicknesses of cuticle, subcuticle, cortex, longitudinal parenchymal muscles, transverse parenchymal muscles, medulla (lateral) medulla (uterine region), in a cross section of a segment 1100–1400 μ thick—20, 140, 100–120, 120, 20, 300, 600 μ respectively. Two sets of genitalia per segment. Testes large, appearing, in a cross section, in a single layer of 12 follicles between each margin and the nearest uterus, and as a double layer of about 12 follicles between the two uteri. Yolk gland layer interrupted opposite the distended uteri by gaps, each one tenth of the segment width. Uteri with 4–8 closely appressed loops on each side, somewhat rosettiform in arrangement. Adults in sea-lions (*Callorhinus ursinus*), St. Paul's Island, Alaska. This type recalled somewhat *Krabbea grandis* recorded by R. Blanchard (1894) from man in Japan.

4. Forms with the scolex similar to the preceding type, broadly oval or heart-shaped and about 1.5 mm wide in marginal outline, narrowly oval in surficial outline (bothrial view). Bothria conspicuous, narrowly triangular, broader anteriorly than posteriorly. No glandular tissue seen within the scolex parenchyma. Neck, in relaxed specimens, short, tending to be hidden by the scolex overhang. Body margins only moderately serrated. Segments mainly broadly rectangular in shape averaging 2.5 mm wide, moderately craspedote, with the cirro-vaginal pores ranging from antero-central to the junction of the first and second thirds. In a cross section of a segment 500 μ thick, the respective thicknesses of cuticle, subcuticle, cortex, parenchymal muscle zone and medulla were 5, 75, 20, 75, 150 μ respectively. Testes, in segment cross section, appearing in single layer, 9–11 follicles lateral to each osmoregulatory canal, 1–2 follicles medial to it. Cirrus pouch 200 μ long, filling the medulla from roof to floor; cirrus often protruded, conspicuous, bluntly lobed, 90 μ long. Yolk gland layer as lateral crescentic bands, interrupted widely mid-dorsally

and mid-ventrally by gaps of one fourth to one fifth of the segment width.   Uterus in our material commonly immature, as a serpentine canal; when mature, with 6–8 loops on each side, somewhat rosettiform in arrangement.   Adults in sea-lions (*Callorhinus ursinus*) St. Paul's Island, Alaska.

The four types outlined above seem to lie within a single generic group which, like many pseudophyllidean generic groups, is highly variable in such details as scolex shape, relative thicknesses of cuticle, subcuticle, cortex, parenchymal muscle zone and medulla, number and arrangement of testes in cross sectional view, mono-gonadism and diplogonadism of genitalia, arrangement of uterine loops, according to the extent of contraction of the specimen and to its state of gonad development. This generic group may be named *Cordicephalus* and defined as follows:

Cordicephalus n. gen. (= *Diplogonoporus* Loennberg, 1892 *ex parte; Krabbea* R. Blanchard, 1894; *Dibothriocephalus* Lühe, 1899 *ex parte; Diphyllobothrium* Lühe, 1910 *ex parte*).   Short thick-bodied forms with the scolex typically heart-shaped, broadly oval or triangular in marginal outline, lanceolate to broadly oval in surficial outline.   Bothria gaping, with prominent margins, commonly wider anteriorly so as to appear in outline like a keyhole or a reversed triangle. Glandular tissue lacking from the scolex parenchyma.   Neck short, wide, commonly overlapped by the scolex.   Body commonly broadening rapidly from the scolex backwards, with the margins markedly serrated.   Segments conspicuously short and craspedote, each with the cirro-vaginal pore in the first third of the ventral surface.   Parenchymal musculature well de-veloped, appearing in a cross section as a continuous conspicuous band, equal to, or one half of, the medullary thickness; genitalia single or double per segment.   Testes relatively few, large spheroidal, appearing in cross section in single or double layer to the number of 10–15 follicles on each side of the uterus.   Cirrus pouch in length two-thirds of the medullary thickness, with the protruding cirrus apically lobed and the external seminal vesicle dorsal and well developed. Ovary with two flattened lobes.   Yolk glands in a cortical band, usually interrupted dorsally and ventrally, opposite the distended uterus, by gaps one tenth to one sixth of the segment width. Uterus with 4–8 loops on each side, closely appressed and somewhat parallel but often loosely rosettiform in arrangement.   Eggs with rounded ends.   Life cycle unknown.   Plerocercoids of northern forms known from marine salmonoid fishes.   Adults in seals and sea-lions, often very numerous in the individual host; sometimes in walruses, and in dogs and humans in seal-frequenting areas (Labrador, Japan, Transbaikalia).   Genotype, *phocarus* Fabricius, 1780.

From Phocidae (seals) in northern Atlantic waters have been recorded:

(a) diplogonadic forms—*tetrapterus* v. Siebold, 1848; *variabilis* Krabbe, 1865; *fasciatus* Krabbe, 1865; these are usually placed in the genus *Diplogonoporus* Loenn-berg, 1892.

(b) monogonadic forms—*phocarum* Fabricius, 1780; *phocae* Mueller, 1780; *phocae-foetidae* Creplin, 1825; *hians* Diesing, 1850; *cordatus* Leuckart, 1863; *elegans* Krabbe, 1865; *lanceolatus* Krabbe, 1865; *schistochilos* Germanos, 1895; *polycal-ceolus* Ariola, 1896; *römeri* Zschokke, 1903; *coniceps* Linstow, 1905; and *macro-phallus,* Linstow, 1895.   The species *minus* Cholodkovsky, 1916, recorded both by Cholodkovsky and by Talysin (1934) from man in Transbaikalia (Russia) would seem also in its features to belong to this group and may be normally a parasite of seals (*Phoca sibirica*) in Lake Baikal.

These forms, though varying in detail, agree in general features with our Types 1 and 2.   The criteria upon which dibothriocephalid species are separated, namely— the shape of scolex, length and breadth of strobila, number and arrangement of testes as seen in a cross section, number of uterine loops, dimensions of intra-uterine eggs— are unreliable at best and especially so when applied to forms so muscular and con-tractile.   We suggest that not only should these forms from northern seals be placed within the genus *Cordicephalus,* but that they are co-specific, belonging within one highly variable species to which the oldest term, *phocarum* of Fabricius, is applicable.

From Phocidae in antarctic waters have been recorded:—*antarcticus* Baird, 1853; *quadratus* Linstow, 1892; *tectus* Linstow, 1892; *scotti* Shipley, 1907; *wilsoni* Shipley, 1907; *coatsi* Rennie and Reid, 1912; *mobilis* Rennie and Reid, 1912; *scoticum* Rennie and Reid, 1912; *clavatum* Railliet and Henry, 1912; *resimum* Railliet and Henry, 1912; *archeri* Leiper and Atkinson, 1914; *lashleyi* Leiper and Atkinson, 1914; and *rufum* Leiper and Atkinson, 1914. Some of the descriptions, notably those provided by Railliet and Henry (1912), Fuhrmann (1921) and Nybelin (1931), are sufficiently detailed to permit an appraisal of the material described. Other descriptions, notably those by Rennie and Reid (1912) and Leiper and Atkinson (1914, 1915) lack detail and refer to material of small size and obvious immaturity. Baird's *Bothriocephalus antarcticus,* which has glandular tissue within the scolex, was selected by Fuhrmann (1921) as the genotype of his suggested genus *Glandicephalus.* To this same genus may be relegated also *mobilis* and *wilsoni.* The species *quadratus, resimum, coatsi* and *scotti*—of *Diphyllobothrium* Lühe— stand apart, from other forms from seals, in having weak parenchymal musculature, recalling in this respect the genus *Dibothriocephalus.*

The remainder have sufficient in common with the northern forms to justify their inclusion within *Cordicephalus* as the species *tectus* Linstow, 1892.

From Otariidae (sea-lions) there have been recorded:—*glacialis* Cholodkovsky, 1914; *pacificus* Nybelin, 1931; *septentrionalis* Nybelin, 1931 (= *Bothriocephalus* sp. of Stiles and Hassall, 1899); *arctocephalinum* Johnston, 1937; and *arctocephali* Drummond, 1937.

Nybelin would place his two forms within a suggested genus, *Adenocephalus* as having glandular tissue within the scolex, and being in other features distinguishable from *Glandicephalus.* The two Australian species, *arctocephalinum* and *arctocephali,* agree closely with our fourth type. The form recorded by Cholodkovsky (1914) as *Clestrobothrium glacialis* from *Callorhinus ursinus* in the Pribylov Islands must be regarded as *species inquirenda.*

It is our considered opinion therefore that Lühe's generic term *Diphyllobothrium* should be discarded, and that the species comprised under it should be distributed among the genera *Diphyllobothrium* Cobbold, 1858; *Cordicephalus auctorum: Diplogonoporus* Loennberg, 1892; *Glandicephalus* Fuhrmann, 1921; *Adenocephalus* Nybelin 1931; *Spirometra* Mueller, 1937; and *Dibothriocephalus* Lühe, 1899, distinguishable as follows:

KEY TO THE GENERA AND SPECIES FORMERLY COMPRISED
UNDER *Diphyllobothrium* LÜHE

1. A. Parenchyma of the scolex with numerous unicellular glands which discharge their contents on the outer and inner surfaces of the bothria 2
   B. Parenchyma of the scolex without such glands 3
2. A. Cirrus pouch connected with the cirro-vaginal atrium by a canal.
   Adenocephalus Nybelin, 1931
   (a) With one species, *pacificus* Nybelin, 1931, with the characteristics of the genus; in *Arctocephalus australis* (Southern Sea-lion), southern Pacific Ocean. ref. Nybelin (1931).
   B. Cirrus pouch opening directly to cirro-vaginal atrium.
   Glandicephalus Fuhrmann, 1921

(a) *antarcticus* Baird, 1853 (= *antarcticum* Diesing, 1863; *antarcticus* Zschokke, 1903; *antarcticus* Shipley, 1907; *antarcticus* Railliet and Henry, 1912); scolex bluntly finger-shaped; bothrial margins gaping widely anteriorly, overlapping posteriorly; uterus with narrow lateral loops and eggs in single file; in *Ommatophoca rossi* (Ross's Seal), Antarctica; ref. Fuhrmann (1921).

(b) *wilsoni* Shipley, 1907; scolex egg-shaped; bothria narrowly slit-like; uterus with terminal loop distended; in *Ommatophoca rossi, Ogmorhinus leptonyx* (Leopard Seal), *Leptonychotes weddelli* (Weddell's seal, Antarctica; refs. Baird, (1853), Shipley (1907), Fuhrmann (1921).

3. A. Scolex heart-shaped or broadly oval in marginal outline, with bothria deep; neck short; commonly hidden; body strongly muscular; in sea mammals (*Pinnipedia, Cetacea*) 4

B. Scolex finger-shaped, spoon-shaped, club-shaped, but rarely heart-shaped; neck prominent; body only weakly muscular; in land mammals (*Carnivora, Primates*) and in fish-eating birds 6

4. A. Very large forms, up to or exceeding 2500 mm long by 15 mm broad; scolex compressed, small, not exceeding one millimeter either way, broadly oval in marginal outline, bluntly pointed apically; with bothrial margins projecting, overlapping, sometimes festooned; uterine loops numerous, parallel, close together, not rosettiform in arrangement; in toothed whales.

Diphyllobothrium Cobbold, 1858

(a) *stemmacephalum* Cobbold; scolex triangular in marginal outline, 160 μ long by 240 μ wide; longitudinal muscle zone two and half times the thickness of the transverse muscle zone; cirrus pouch 320–370 by 160–270 μ; 12–15 uterine loops on each side; eggs 55 by 40 μ; adults in *Delphinus phocaena,* northern Atlantic waters; refs. Cobbold (1858), Cohn (1912).

(b) *fuhrmanni* Hsü, 1935 (= *stemmacephalum* of Yamaguti, 1935); scolex elongated in marginal outline, 250 by 240 μ; longitudinal muscle zone five to six times the thickness of the transverse muscle zone; cirrus pouch 640 by 260 μ; 7–9 uterine loops on each side; eggs 63–66 by 45–47 μ; adults in *Delphinus dussumieri* and *Neomeris phocaenoides* Sino-Japanese waters; refs. Hsü (1935), Yamaguti (1935).

B. Small to large forms with scolex typically heart-shaped in marginal outline; segments short, broad, often with double genitalia; uterine loops few and tending to be loosely rosettiform in arrangement 5

5. A. Relatively large forms with bothria deep and slit-like; segments very short and broad each with double genitalia; uterine loops few and rosettiform in arrangement; adults in whalebone whales .. *Diplogonoporus* Loennberg, 1892

(a) *balaenopterae* Loennberg, 1892; with the characters of the genus; each uterus with 4–5 loops on each side; eggs 67 by 42 μ; in *Balaenoptera borealis,* Scandinavian waters; ref. Loennberg, 1892. A doubtful form which may yet find its true position in *Cordicephalus*.

B. Relatively short, broad forms with scolex typically heart-shaped in marginal outline, oval or triangular in surficial outline; bothria deep, keyhole-like; genitalia typically single but often double; uterus with 4–8 loops on each side, usually parallel and close together, sometimes loose and rosettiform; end loop greatly distended; eggs with rounded ends; in seals, sea-lions, walruses; occasionally in humans and dogs .... *Cordicephalus auctorum*

    (a) *phocarus* Fabricius, 1780 (= *phocarum* Fabricius, 1780; *phocae* Muller, 1780; *phocae foetidae* Creplin, 1825; *tetrapterus* v. Siebold, 1848; *hians* Diesing, 1860; *cordatus* Leuckart, 1863; *elegans* Krabbe, 1865; *lanceolatus* Krabbe, 1865; *variabilis* Krabbe, 1865; *schistochilos* Germanos, 1895; *polycalceolus* Ariola, 1896; *römeri* Zschokke, 1903; *macrophallus* Linstow, 1905; *coniceps* Linstow, 1905; *minus* Cholodkovsky, 1916) ; with scolex typically heart-shaped or triangular in marginal outline; body broadening rapidly from scolex backwards, with margins markedly serrated; segments markedly craspedote; genital pore, or pores, at junction of first and second thirds of ventral segment surface; in contracted segments commonly hidden by overlap of preceding segment; uterine loops 3 or 4 on each side, close together, parallel, with terminal loop greatly distended and just level with cirrus pouch; adults in seals of northern Atlantic waters, occasionally in dogs, humans; refs. Stunkard and Schoenborn, 1936; Lyster, 1940.

    (b) *tectus* Linstow, 1892 (= *scoticum* Rennie and Reid, 1912; *perfoliatum* Railliet and Henry, 1912; *clavatum* Railliet and Henry, 1912; *lashleyi* Leiper and Atkinson, 1914) ; small, muscular forms, up to 220 mm long by 7 mm broad; scolex elongated, finger-shaped or club-shaped; bothria keyhole-like; neck short but usually visible.; segments short, moderately craspedote; genital pore at junction of first and second thirds (relaxed) or near anterior border (contracted) of ventral segment surface; uterine loops "relatively few"; adults in seals of antarctic waters; refs. Linstow (1892), Railliet and Henry (1912), Rennie and Reid (1912), Leiper and Atkinson (1914–1915).

    (c) *arctocephalinus* Johnston, 1937 (= *arctocephali* Drummond, 1937; *septentrionalis* Nybelin, 1931; *glacialis* Cholodkovsky, 1914; *grandis* R. Blanchard, 1894) ; relatively long, slender forms with scolex up to 2 mm long, heart-shaped in marginal outline, oval in superficial outline; bothria narrowly triangular, broader anteriorly than posteriorly; neck short, but visible; segments mainly rectangular, moderately craspedote; genital pore or pores antero-central (relaxed) or at junction of first and second thirds (contracted) ; genitalia single, occasionally double; uterus with 4–5 loops on each side; loosely arranged; somewhat rosettiform; uterus mainly or even entirely behind the cirrus pouch; adults in sea-lions *Callorhinus ursinus, Arctocephalus australis, A. forsteri, A. tasmanicus*), Alaskan and Australasian waters; refs. Johnston (1937), Drummond (1937).

(d) *quadratus,* 1892 (= *resimum* Railliet and Henry, 1912; *coatsi* Rennie and Reid, 1912) ; small forms with scolex egg-shaped; parenchymal muscles weak; uterus with 6 or 7 loops on each side, becoming distended terminally ; genital pore at junction of first and second fifths; adults in *Ogmorhinus leptonyx* (Leopard Seal), Antarctica; refs. Linstow (1892), Railliet and Henry (1912), Rennie and Reid (1912), Fuhrmann (1921).

The "relatively weak parenchymal musculature" (Fuhrmann) throws doubt upon the admissibility of this form to the genus *Cordicephalus.* Fuhrmann's re-description was based upon Linstow's original material which may not in this respect have been typical. The other characteristics are clearly cordicephalid.

6. A. Small to medium sized, weakly muscular forms, with the scolex small, compressed, in marginal outline spoon-shaped or finger-shaped; bothria slitlike but broad and shallow and fading indefinitely into the mid-dorsal and mid-ventral furrows of the body ; neck long and slender ; cirrus and vagina opening independently upon a slight swelling of the ventral segment surface caused by the underlying cirrus pouch ; cirrus pouch composite, its dorsal portion representing the external seminal vesicle of other genera ; uterus a simple spiral of closely appressed coils, never rosettiform in arrangement ; eggs pointed at each end ; adults mainly in cat-like *Carnivora,* occasionally in dogs and humans . . . . *Spirometra* Mueller, 1937

    (a) Lateral bands of testes and yolk glands uniting in front of the genital pores; *bresslauei* Baer, 1927; *decipiens* Gedoelst, 1911; *erinacei* Faust et al, 1929; *felis* Southwell, 1928; *gracile* Baer, 1927; *houghtoni* Faust et al, 1929; *mansoni* of Joyeux (1927) ; *mansonoides* Mueller, 1935; *reptans* of Joyeux and Houdemer (1928) ; *serpentis* Yamaguti, 1935; and *urichi* Cameron, 1936.

    (b) Lateral bands of testes and yolk glands not uniting in front of the genital pores; *okumurai* Faust et al, 1929 ; *pretoriensis* Baer, 1924; *raillieti* v. Ratz, 1913. (= *erinaceieuropaei* of Brumpt, 1936) ; *ranarum* Meggitt, and *reptans* of Meggitt (1924).

Incertae sedis: tangalongi MacCallum, 1921.

B. Relatively large, slender, weakly muscular forms with the scolex compressed, elongated, in marginal outline olive-shaped, spoon-shaped, club-shaped, according to age and degree of contraction ; in surficial outline narrowly oval or lanceolate ; bothria narrow, deep, slit-like, without projecting margins; neck long and slender ; body only weakly craspedote and serrated ; cirrus and vagina opening into a common cirro-vaginal atrium; genital pore in first third of ventral segment surface ; uterus with 4–8 loops on each side, somewhat crowded and twisted, pointing forwards or backwards in rosette fashion ; eggs with rounded ends ; adults in dog-like mammals and in fish-eating birds . . . . *Dibothriocephalus* Lühe, 1899

    (a) In mammals: *latus* Linnaeus, 1758; *laruei* Vergeer, 1942; *theileri* Baer, 1925; *trinitatus* Cameron, 1936.

    (b) In birds: *canadensis* Cooper, 1921 ; *cordiceps* Leidy, 1871; *dendriticus* Nitzsch, 1824; *oblongatus* Thomas, 1946.

Dubia or *inquirenda: americanus* Hall and Wigdor, 1918; *fuscus* Krabbe, 1865; *nenzi* Petrov, 1938; *parvus* Stephens, 1908; *similis* Krabbe, 1865; *skrjabini* Plotnikov, 1932; *strictus* Talysin, 1932 (similar to `oblongatus` according to Thomas, 1946) ; *taenioides* Leob, 1930; *tungussicus* Podjapolskaya and Gnedina, 1932; all from mámmals: *ditremus* Creplin, 1825 (but *vide* Baylis, 1945) ; *exile* Linton, 1892; and *fissiceps* Creplin, 1829, from birds.

The species of *Spirometra* and *Dibothriocephalus* cannot readily be separated upon a few dogmatically stated characteristics but must be identified from the whole picture of morphological and distributional and, if possible, life-cycle data.

SUMMARY

A new genus, *Cordicephalus,* is established for those species of *Diphyllobothrium* Lühe, 1910, found in seals and sea-lions, with four species, *phocarus* Fabricius, 1780; *tectus* Linstow, 1892; *arctocephalinus* Johnston, 1937; and *quadratus* Linstow, 1892. The remaining species of Lühe's genus are distributed between the genera *Diphyllobothrium* Cobbold, *Diplogonoporus* Loennberg, *Glandicephalus* Fuhrmann, *Adenocephalus* Nybelin, *Spirometra* Mueller, and *Dibothriocephalus* Lühe.

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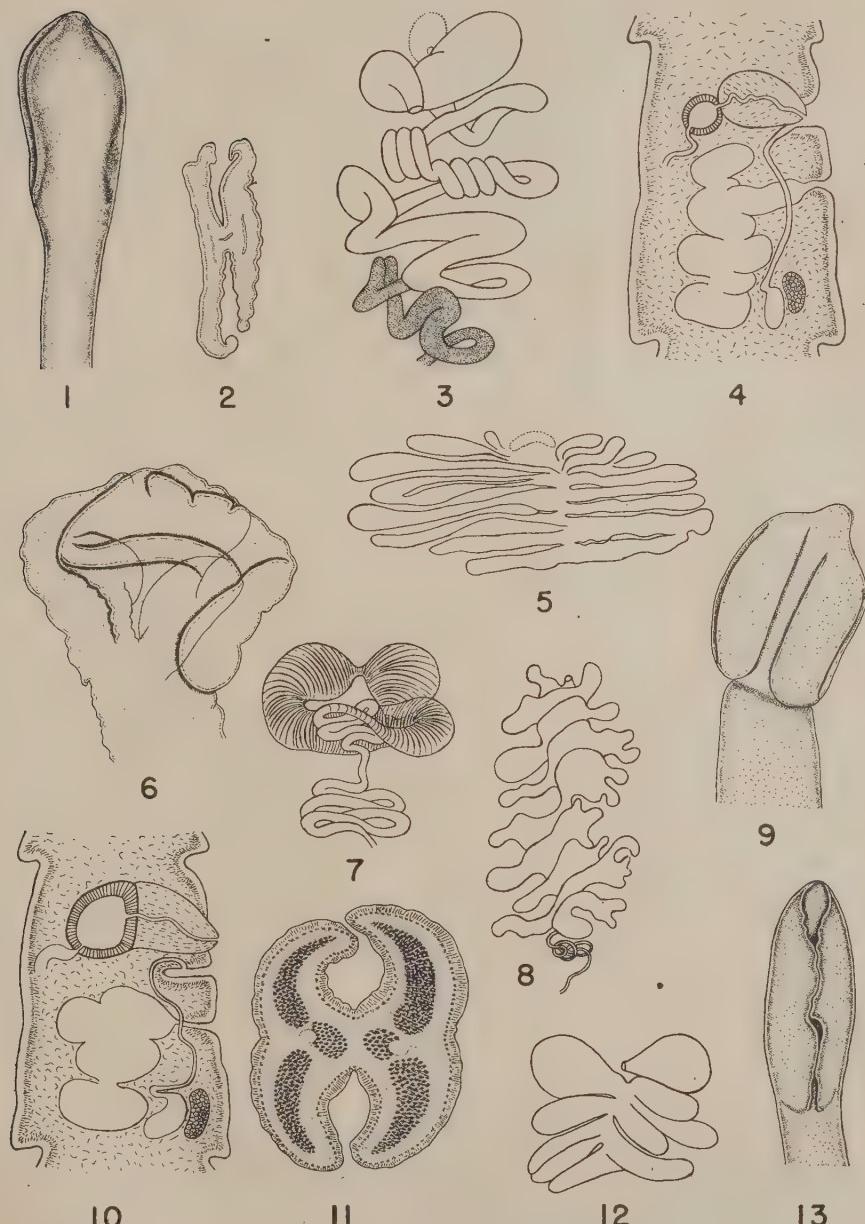
FIGS. 1, 2, 3. *Dibothriocephalus* after Wardle & McColl, 1937. FIG. 4. *Dibothriocephalus* after Mueller, 1937. FIGS. 5, 6. *Diphyllobothrium* after Yamaguti, 1935. FIGS. 8, 9. *Diphyllobothrium* after Hsü, 1935. FIGS. 7, 10. *Spirometra* after Mueller, 1937. FIGS. 11, 12, 13. *Adenocephalus* after Nybelin, 1931.
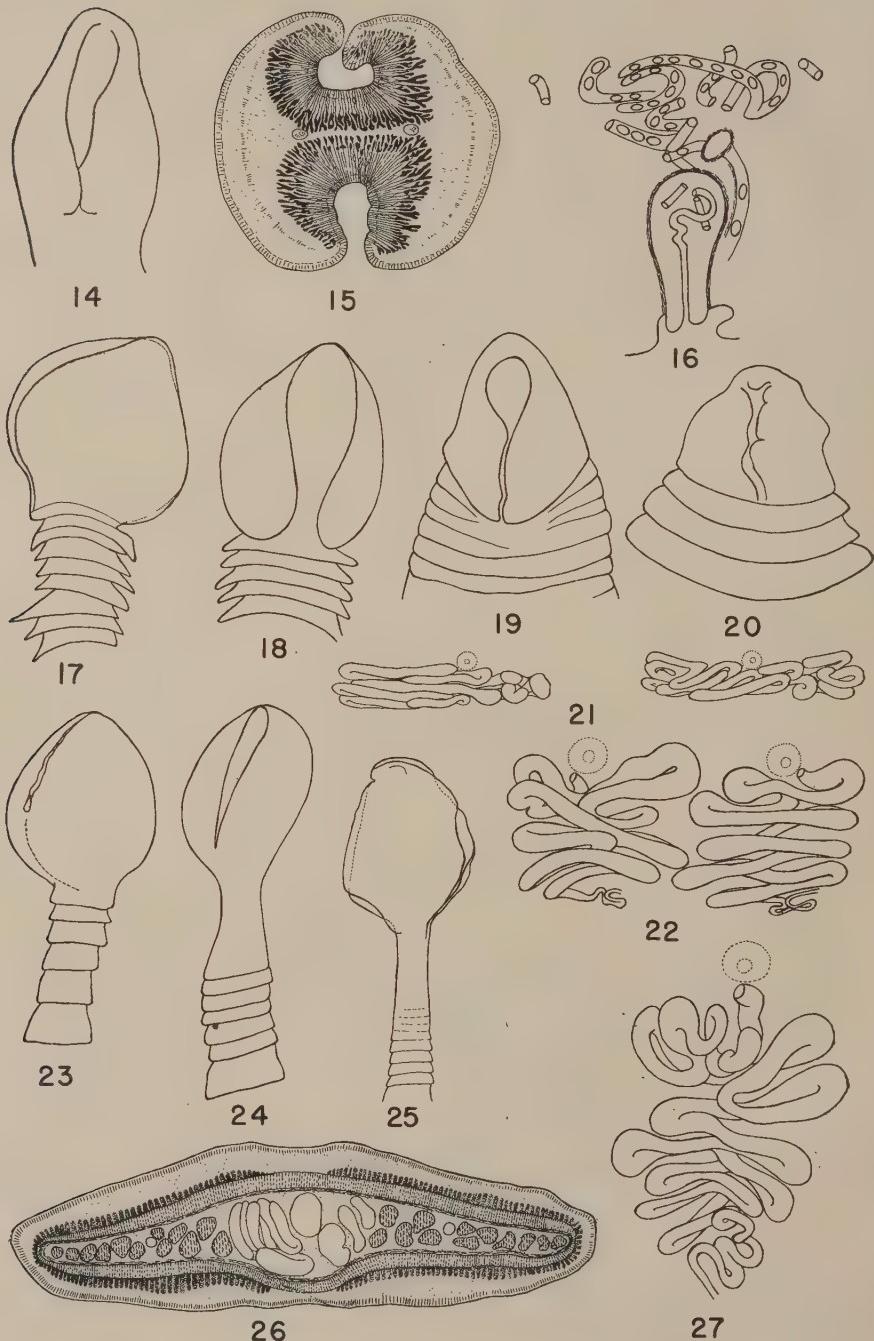
FIGS. 14, 15, 16.   *Glandicephalus* after Fuhrmann, 1921.   FIGS. 17–21.   *Cordicephalus phocarus.*
FIGS. 22–27.   *Cordicephalus arctocephalinus.*

THE THERMAL DEATH POINT OF CYSTICERCI OF
TAENIA SAGINATA

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INTRODUCTION

Although it has long been recognized that thorough cooking of beef will destroy contained cysticerci of *Taenia saginata,* the larval stage of the common tapeworm of man, opinions of investigators concerning exact temperatures that are lethal to this parasite are somewhat varied. For example, Lewis (1872) heated "in fresh water, in salty water, and in a dry state" encapsulated cysticerci to which a small amount of meat was left attached, and reported that death of the parasite, as evidenced by lack of movement, resulted when the organisms were exposed to a temperature of 54.4° C, for 5 minutes. Pellizzari et al (1874) reported that cysticerci die when heated to 60° C. Perroncito (1877) exposed decapsulated cysticerci to various temperatures and reported, mainly on the basis of the appearance of the parasites and whether or not movement could be induced, that they sometimes die at 44° C, often at 45° C, and never survive exposure to a temperature of 46° C. In some cases the cysticerci, after subjection to heat, were swallowed by human subjects to ascertain whether the parasites were alive. Ostertag (1913) stated that cysticerci will not survive temperatures of 45° to 50° C. Stiles (1898) reported that according to Hertwig a temperature of 52° C reduces the parasites to a smeary, soft condition. Clarenburg (1931) immersed pieces of muscle tissue, about 8 cm thick, containing cysticerci in boiling water for periods of 5, 10, 15, 20, 30, and 50 minutes and used the ability of the cysticerci to evaginate in vitro as a criterion of life. While evagination did occur in those heated 10 minutes or less, those heated for longer periods failed to evaginate, and were, therefore, considered dead. The internal temperatures of the pieces of meat in which the cysticerci were killed varied from 71° to 91° C. The internal temperatures of those pieces in which the cysticerci were not killed were not specified.

To obtain specific information on the exact temperatures at which cysticerci of *Taenia saginata* die, a series of tests was carried out in which (a) decapsulated cysticerci, and (b) small pieces of flesh containing the encapsulated parasites, were heated to various temperatures in physiologic saline or Ringer's solution and the viability of the parasites tested (1) by exposure to warm solutions of sodium taurocholate (sodium salt of a bile acid, taurocholic acid), to induce movement and/or evagination, (2) by observance of flame cells for evidence of activity in those cysticerci that failed to exhibit movement or that failed to evaginate in the warm sodium taurocholate solution, or (3) by passage through the digestive tract of a human subject.

MATERIALS AND METHODS

Cysticerci used in the experiments recorded in this paper were obtained from the flesh of four naturally-infected bovines ranging in age from about 5 months to approximately 5 years. The infections harbored by the host animals in question were

discovered in routine post-mortem examinations conducted by workers in the Federal meat inspection service. The carcasses were stored at a temperature of approximately 4° C until needed. The intervals which elapsed after slaughter of the animals and before the cysticerci were tested ranged from a few hours to 12 days.

Method of heating decapsulated cysticerci.—The cysticerci were first removed from their capsules by slicing the flesh thinly with a sharp knife. As they were recovered, they were placed in physiologic saline or Ringer's solution and held at room temperature. From each lot of cysticerci removed from the meat, 5 to 8 were selected at random and kept in physiologic saline or Ringer's solution maintained at room temperature during the longest period that the test organisms were exposed to heat; these cysticerci served as controls.

For exposure to heat, cysticerci were placed in 25 cc of Ringer's solution or physiologic saline in each of a series of 100-cc beakers. The beakers were then placed in a water bath which consisted of a 1,000-cc beaker containing about 800 cc of water. Throughout the heating period the temperature of the water bath was held, by means of a Bunsen burner, at a point 2° C higher than that to which the parasites were to be exposed. This 2° difference in temperature permitted a uniform rise in the temperature of the liquid in the test beakers that could be measured accurately. During the heating period, the beakers were frequently agitated and when the medium surrounding the cysticerci reached the desired temperature the beakers were removed from the water bath and to their contents about 50 cc of cool physiologic saline or Ringer's solution (temperature 4° to 20° C) were immediately added. This served to lower rapidly the temperature of the liquid surrounding the cysticerci. Viability tests were initiated immediately.

Method of heating cysticerci encapsulated in muscle tissue.—In most cases, pieces of heart and tongue weighing from 25 to 40 grams were utilized; a thermometer bulb was inserted into the center of each. Actual measurements of all the individual pieces were not made but representative pieces measured from 3 to 5 cm in length and from 2 to 4 cm in thickness. For heating, each piece was submerged in physiologic saline, the temperature of which was maintained throughout the heating period at the temperature to which the meat was to be heated. In one instance (Table 2), 10 pieces of muscle tissue too small to hold a thermometer were heated. The maximum thickness of these pieces was about 12 mm, and their average weight was 6.2 grams. Heat was applied in a manner similar to that used in heating decapsulated cysticerci; that is, the pieces were placed in physiologic saline in a small beaker which was held in the water bath. The recorded temperature in this case was that of the saline surrounding the muscle tissue.

As soon as the desired temperature had been attained in the center of the pieces of muscle tissue being heated, they were removed from the water bath and immediately plunged into physiologic saline having a temperature of 4° to 20° C. This served to arrest the temperature rise and to cool the meat to well below body temperature. The cysticerci were recovered by slicing the meat thinly with a sharp knife. Viability tests were then carried out.

Testing the viability of the cysticerci.—Tests of the viability of the cysticerci exposed to heat and of appropriate unheated control cysticerci were carried out by (1) heating in fresh 0.6 per cent solution of sodium taurocholate in physiologic saline or Ringer's solution (temperature 37° C) using a modification of the techniques de-

scribed by Malkani (1933) and Edgar (1941), or (2) passage through the alimentary tract of a human subject, utilizing a modification of the techniques described by Keller (1935) and Viljoen (1937). Cysticerci to be exposed to the sodium taurocholate solution were first examined for evidence of evagination. This was necessary because it was observed that during some of the test exposures to heat a small number of cysticerci became partially evaginated. It was necessary, therefore, to differentiate changes that took place in cysticerci before they were subjected to the sodium taurocholate solution from those that occurred in the solution.

After the preliminary examination, cysticerci were placed in 25 cc of the fresh taurocholate solution (temperature 37°C) contained in 100-cc beakers. In most cases, not more than 6 cysticerci were placed in each 25 cc of solution. The beakers were held in a water bath, the temperature of which was adjusted to keep the temperature of the taurocholate solution at 37° C. During the period the cysticerci were being exposed to the testing solution the beakers were agitated frequently. Control cysticerci were always tested at the same time and in part of the same lot of solution as was utilized for tests on the cysticerci exposed to heat.

Examinations for changes indicative of evagination were made with the aid of the low power of a dissecting microscope at intervals of about 15, 30, 60, and 120 minutes after the beginning of the testing period. Cysticerci found to be completely evaginated were removed from the solution as soon as this condition was observed; all others were held in the solution for 2 hours. The changes which took place in the cysticerci are recorded in the tables as "complete" or "partial" evaginations. Complete evaginations were those in which the suckers were plainly visible; anything less was classed as partial evagination.

Cysticerci which remained inactive during exposure to the taurocholate solution were examined for flame cell activity. Schmey and Bugge (1931) used this method extensively in their investigations of the effect of refrigeration on this parasite. In making the observations in the present experiments the scolex of the parasite was teased apart from the remainder of the cysticercus and examined under oil immersion. This method never failed to demonstrate active flame cells in control cysticerci. Those cysticerci in which flame cells were active were considered alive even though evagination had not occurred; when the flame cells were inactive the parasite was considered dead.

As stated previously, tests of the viability of some cysticerci were made by passing them through the alimentary tract of a human subject, utilizing a modification of the techniques described by Keller (1935) and Viljoen (1937). The technique used is briefly as follows:

Glass tubes made from ordinary glass tubing having a bore of 3 mm were used. Each tube was cut to a length of about 13 mm and the ends fire polished and flared. When more than one tube was used they were marked with a diamond pencil for identification. Cysticerci that were tested by this method were not tested in taurocholate solution or observed for flame cell activity. They were transferred directly to the tubes from the physiologic saline or Ringer's solution in which they were stored after removal from the host tissue. It was found that the fluid adhering to the cysts was sufficient to keep them moist inside the tube. After inclusion of the cysticerci the tubes were wrapped in square pieces of silk cloth, the corners of which were brought together, twisted, and sewn securely with cotton thread, care being

taken to see that only one layer of the cloth covered the ends of the tube. After passage through the digestive tract of the human subject, the tubes were recovered from the feces, washed and placed in alcohol, where they were held until the contained cysticerci could be examined.

EXPERIMENTAL FINDINGS

The following brief presentation of the data will serve to point out some of the more important facts elicited by the investigation.

Decapsulated cysticerci.—As shown in Table 1, decapsulated cysticerci were heated to temperatures of 50°, 53°, 54°, 55°, and 56° C. The periods of time that elapsed in attaining the temperatures in question ranged from 3 1/3 to 5 1/2 minutes. Cysticerci heated to 50° C were apparently unaffected as they evaginated almost as

TABLE 1.—*Thermal death point of decapsulated cysticerci of* Taenia saginata *heated gradually in physiologic saline or Ringer's solution*

Cysticerci*	Temperature attained	Time in bath	In vitro tests of viability of cysticerci				Keller tests of viability of cysticerci		
			No. of cysticerci	No. of evaginations		Motility of flame cells	No. of cysticerci	Recovery time	Results
				Complete	Partial				
	°C	minutes						hours	
A7	50	4½	5	1	4	No observation	..	..	..
A7	53	3¾	5	0	5	do	..	..	..
B2	53	4½	5	0	0	Active	..	..	..
B2	53	3½	5	0	2	do	..	..	..
A8	54	4	5	0	0	Inactive	..	..	..
B2	54	5½	5	0	2	Active	..	..	..
B2	54	4⅙	5	0	2	Inactive	..	..	..
A8	55	5	5	0	0	do	..	..	..
B2	55	4⅙	5	0	0	do	..	..	..
B2	55	5½	5	0	3	do	..	..	..
B6	55	4¾	5	0	0	do	..	..	..
B6	55	3¾	5	0	1	do	..	..	..
A8	56	5	5	0	0	do	..	..	..
A7	56	4½	3	0	0	No observation	2	19	Dead
B6	56	3¾	5	0	0	Inactive	..	..	..
B8	56	4¼	5	0	0	do	..	..	..
B10	56	4¾	4	0	0	do	2 ⎤		
B10	56	3½	4	0	0	do	2 ⎬	28	Dead
B10	56	4	4	0	0	do	2 ⎦		
C4	56	5	5	0	0	do	2	19	do
D0	56	4	44	0	0	No observation	..	..	..
D1	56	4†	38‡	0	0	5 examined, inactive	5	64	Dead
A7	Control, not heated		8	1	7	No observation	3	40	Dead
A8	do		7	2	5	do	..	..	..
B2	do		5	4	1	Active	..	..	..
B2	do		7	0	7	do	..	..	..
B6	do		5	5	0	No observation	..	..	..
B8	do		5	0	5	Active	..	..	..
B10	do		5	1	4	do	..	..	..
B12	do		..	..	..	No observation	5	22	Alive
B13	do		..	..	..	do	2	19	do
B13	do		..	..	..	do	3	44	Dead
C4	do		5	5	0	Active	..	..	..
D0	do		5	5	0	No observation	..	..	..
D1	do		5‡	4	1	Active	..	..	..

* Obtained from four naturally infected bovine hosts aged approximately 5 months, ——————, ——————, and 5 years when slaughtered. These 4 hosts are referred to as A, B, C and D in the first column of the table, and the figure following each letter designates the number of days from the death of the host to the beginning of the test.
† Heated in Ringer's solution.
‡ Bile salt solution made up with Ringer's instead of saline.

quickly in the bile salt solution as did the unheated controls. It was not considered necessary, therefore, to make observations on the flame cells in these larvae. Of those heated to 53° and 54° C, some evaginated partially; the others had active flame cells. Twenty-five cysticerci were heated to 55° C; at the end of 2 hours in the

taurocholate solution 4 were partially evaginated, but active flame cells were not observed in any of the parasites heated to this temperature. One hundred thirty-two cysticerci were heated to a temperature of 56° C. Of these, 117 were tested in taurocholate solution, but none of them evaginated and movement was not observed. Of the 117 cysticerci, 37, selected at random, were examined for flame cell activity but none was observed. The remaining 15 cysticerci were tested by the Keller method, but no evaginations occurred. These facts show that the parasites had been killed by the temperature employed.

Cysticerci encapsulated in flesh.—Pieces of host tissue containing cysticerci were heated to internal temperatures of 45°, 50°, 55°, 56°, and 57° C. (See Table 2.) In the test involving a temperature of 45° C, a piece of tongue weighing 35 grams was utilized and 18 minutes was required to raise the internal temperature to the point named. Ten cysticerci were recovered from the sample in question. All be-

TABLE 2.—*Thermal death point of cysticerci of* Taenia saginata *in small pieces of muscle tissue heated gradually in physiologic saline*

Cysticerci*	Weight of meat	Time in water bath	Internal temperature attained	In vitro tests of viability of cysticerci			Motility of flame cells	Keller tests of viability of cysticerci		
				No. of cysticerci	No. of evaginations			No. of cysticerci	Recovery time	Results
					Complete	Partial				
	grams	min- utes	° C						hours	
B3	35	18	45	10	10	0	Active	..	..	..
B3	35	21	50	9	1	8	do	..	..	..
B3	34	18½	55	10	0	3	Inactive	..	..	..
B5	32	18	55	8	0	0	do	2 ⎤		
B5	32	23	55	7	0	0	do	2 ⎬	19	3 alive
B5	32	24½	55	5	0	0	do	2 ⎦		
C2	25	14	56	2	0	0	do	..	..	..
·C2	29	15	56	5	0	0	do	2 ⎱	19	Dead
C2	34	18¾	56	6	0	0	do	1 ⎰		
C2	35	25	56	7	0	0	do	..	..	..
C4	36	23½	56	5	0	0	do	..	..	..
C4	40	32	56	4	0	0	do	..	..	..
C12	27	16	56	5	0	0	do	2 ⎱	43	Dead
·C12	30	19	56	6	0	0	do	1 ⎰		
B8	25	13½	57	3	0	0	do	1 ⎱	22	do
B8	25	15⅔	57	2	0	0	do	1 ⎰		
B9	..†	16¼	57	12	0	0	do	4	46	do
B3		Control not heated	..	8	8	0	Active	4	22	Alive
B5		do	..	8	8	0	do	3	43	do
B8		do	..	5	0	5	do	..	..	..
B9		do	..	7	5	2	do	..	..	..
C2		do	..	5	5	0	do	..	..	..
C4		do	..	5	5	0	do	..	..	..
C12		do	..	5	4	1	do	..	..	..
C14		do	..	..	..	..	No observation	3	22	Alive

* See footnote, Table 1.
† This sample consisted of 10 pieces of masseter muscle, each with a maximum thickness of approximately 12 mm, and with an average weight of 6.2 g. Temperature attained was that of the physiologic saline containing these small pieces.

came completely evaginated in the taurocholate solution, showing that the temperature utilized had not materially affected the viability of the parasites. A 35-gram sample was heated to an internal temperature of 50° C in 21 minutes. Of the 9 cysticerci recovered, one evaginated completely. Although the other 8 parasites failed to become completely evaginated, flame cell activity was observed in all.

Four pieces of tongue were heated to an internal temperature of 55° C. The time required to attain this temperature varied from 18 to 24 1/2 minutes. The 4 pieces

contained a total of 36 cysticerci. Thirty of these were tested in taurocholate solu-
tion and of these, only 3 evaginated partially; however, no active flame cells were
observed in any of the 30 organisms. Six cysticerci were tested in one tube by the
Keller method. When the tube was recovered, 3 of them had not been completely
digested and it was considered, therefore, that they were alive at the time they were
swallowed. The other parasites enclosed in the tube were completely digested and
it was considered that they had been dead at the time they were swallowed.

Eight pieces of tongue, varying in weight from 25 to 40 grams, were each heated
to an internal temperature of 56° C in from 14 to 32 minutes. A total of 46 cysti-
cerci were recovered from the samples. Forty were tested in taurocholate solution.
Evagination did not occur and the flame cells were inactive showing that the
cysticerci had been killed by the temperature employed. The remaining 6 organisms
were completely digested when subjected to the Keller test, which substantiates the
findings of the in vitro test.

Two 25-gram samples of heart muscle were each heated to an internal temperature
of 57° C. One sample required 13 1/2 minutes to reach the temperature indicated;
the other required 15 2/3 minutes. Of the 7 cysticerci recovered from these samples,
5 were tested in taurocholate solution and then observed for flame cell activity. No
evagination occurred and no flame cell activity was observed. One cysticercus from
each sample was tested by the Keller technique and both were found to be com-
pletely digested. These findings show that the cysticerci had been killed by the
temperature employed.

Ten pieces of masseter muscle were placed in physiologic saline which was heated
to 57° C in 16 1/4 minutes. Sixteen cysticerci were taken from these samples; 12
were tested in taurocholate solution. The parasites remained inactive and ex-
aminations were made, therefore, of the flame cells, but no activity was observed.
The 4 remaining cysticerci were tested by the Keller technique and the parasites were
found to be dead. However, the results of the Keller test in this instance, and in one
instance involving 3 cysticerci from muscle tissue heated to 56° C, may be of
questionable validity for reasons discussed later.

DISCUSSIONS AND CONCLUSIONS

Several investigators have emphasized the importance of using only fresh ma-
terial in experiments involving cysticerci. In view of this fact, there has been in-
corporated in the tables for each lot of cysticerci, information concerning the time
between death of the host animal and the beginning of the test. Although the tests
were carried out as rapidly as possible, as much as 12 days elapsed after death of some
host animals before cysticerci recovered from their flesh were tested. It is of interest,
therefore, to consider the possible influence of this elapsed time on the reactions of
the cysticerci in taurocholate solution as reflected by the results from the 3 different
viability tests with the controls. On the basis of the number of complete evagina-
tions, as recorded in Tables 1 and 2, it appears that cysticerci tested within a few
hours after slaughter of the host animal were somewhat more vigorous than those
tested several days after death of the host. In the heating tests this factor, however,
was partly compensated for by using cysticerci from several animals in each experi-
mental group. The tables show that not a single control (unheated) cysticercus
failed to evaginate, at least partially, regardless of the time elapsed since death of the

host. In regard to observations on flame cell activity and the Keller test, there was no indication that the results of either were affected by the period of time that elapsed between the death of the animal and the removal of the cysticerci from the flesh for testing.

The longest time between death of the host and heating of decapsulated parasites was 10 days; in the case of cysticerci heated in muscle the longest elapsed time was 12 days. In each instance, as shown in the respective tables, control cysticerci showed active flame cells on the corresponding days and the activity observed appeared to be just as much in evidence at those times as it was in cysticerci tested at shorter intervals following slaughter of the host. In Table 2, along with the results of the control tests, are given data which show that by the Keller test the results were still positive after 14 days.

In evaluating the results of the Keller test two factors deserve consideration. The first of these is the possibility that cysticerci are not sufficiently exposed to the digestive juices, so that those which are dead when swallowed may pass through the digestive tract with little change. It is probable that such a thing occurred in the present tests in the case of cysticerci heated in muscle to 55° C (see Table 2). On the occasion in question, 6 cysticerci were placed in one tube and when it was recovered from the feces, 3 of the parasites had not been completely digested, and 1 of the 3 still retained its terminal vesicle. Terminal vesicles were never found in the case of control cysticerci after their passage through the digestive tract, so the presence of the aforementioned one may be taken as evidence that there was insufficient exposure to digestive juices, possibly because of too much crowding.

Of more importance is a second factor applying to the Keller test and that is the possibility that the tubes may remain in the digestive tract for so long a time that even cysticerci which are alive when swallowed will be completely digested. On the basis of the results with control cysticerci, it is considered that data pertaining to heated cysticerci are valid only in those instances where the tubes were recovered within 22 hours. However, the fact that Keller (1935), in his work with decapsulated parasites, consistently found undigested cysticerci in tubes recovered within 33 hours lends support to the validity of the findings in a test in which a tube containing 6 cysticerci which had been heated to 56° C (see Table 1) was recovered from the feces after 28 hours. Results from all tubes requiring more than 28 hours to pass through the digestive tract are therefore considered to be of questionable significance in the present investigations.

The methods used by the previous investigators in heating cysticerci make difficult a comparison of their results with those obtained in the present experiments. Lewis (1872) reported only that exposure to a temperature of 125° F (51.6° C) for 5 minutes does not kill cysticerci, while exposure to a temperature of 130° F (54.4° C) for the same length of time does kill them. Perroncito (1877), on the other hand, recorded the appearance of decapsulated cysticerci through a wide range of temperatures but he supplied little information concerning the time required to attain such temperatures. Also, it is apparent that at least some of the cysticerci were subjected to heat in an evaginated state. It is possible that these two factors account for the wide discrepancy between Perroncito's results and those obtained in the present work.

SUMMARY

1. A series of experiments was carried out to determine the thermal death point of cysticerci of *Taenia saginata*. After the cysticerci were exposed to various temperatures, attained gradually, they were tested for viability by the use of one or all of the following criteria: (1) ability to evaginate in warm sodium taurocholate solution, (2) activity of flame cells, and (3) the ability to pass through the digestive tract of man without being digested.

2. Decapsulated cysticerci heated to temperatures as high as 54° C evaginated partially in sodium taurocholate solution and showed active flame cells in some cases. A small percentage of those heated to 55° C evaginated partially in taurocholate solution but none showed active flame cells. Those heated to 56° C did not respond in taurocholate solution, did not show active flame cells, and were completely digested when passed through the digestive tract of a human subject.

3. Cysticerci that were heated in muscle tissue to temperatures as high as 50° C evaginated in taurocholate solution and showed active flame cells. A small percentage of those heated to 55° C evaginated partially but none showed active flame cells. However, 3 out of 6 were not completely digested when passed through the digestive tract of a human subject. Cysticerci heated gradually in muscle tissue to temperatures of 56° C, and those that were heated to 57° C, did not respond in taurocholate solution, did not show active flame cells, and were digested when passed through the digestive tract of a human subject.

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THE RÔLE OF REDUCED FOOD INTAKE IN ALCOHOLIC DEBILITATION OF MICE INFECTED WITH *HYMENOLEPIS**

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Previous studies (Larsh, 1945, 1946) have shown that alcohol, especially in high concentrations given over long periods, reduces the resistance of young mice both to initial infection and to reinfection with *H. nana* var. *fraterna*. Since the alcoholic animals in these experiments appeared to consume much less food than the controls, there was a suggestion that malnutrition may have been an important factor in producing the weakened resistance. The first objective in the present study was to obtain further evidence on the relation of the reduced food intake produced by alcoholic debilitation of mice to the reduction of their resistance to initial infection with *Hymenolepis*. The second objective was to see what effect supplemental vitamins would have on the reduced resistance to this parasite produced by alcoholic debilitation.

EXPERIMENTAL RESULTS AND DISCUSSION

1. *The relationship of food intake and natural resistance to* Hymenolepis.—Most of the experimental details have been given or referred to in the previous work mentioned above, the remainder are given in the description of experiments which follows.

In initial tests with a few animals in each group observed for one week, it was found that mice each given 0.4 cc of 40 per cent alcohol in daily oral injections consumed much less food than the controls. In order to determine the effect on resistance of this diminished food intake, tests were designed to compare alcoholic mice with non-alcoholic controls that consumed about the same amount of food during the treatment period. To do this, it was necessary to know how soon after drug injection the alcoholic mice return to food, and how much food they consume per day.

Preliminary tests showed that young mice two months old given an initial dose by mouth of 0.2 cc of 40 per cent alcohol did not partake of food thereafter for eight hours or longer. The drug volume was increased gradually each day until the maximum dose of 0.4 cc was given for the first time on the fifth day. Two days later, at the end of one week of treatment, all of the animals refused food for 10–13 hours after receiving alcohol. This fasting period following injection of the drug reached its maximum after two weeks of treatment when most of the mice remained away from food for 11–14 hours. Because of the obvious difficulty of establishing the exact feeding period for each mouse, it was decided in the experiments to remove food from all of the non-alcoholic fasting controls for 15 hours daily, i.e., from 5:00 PM when the alcoholic mice were injected until 8:00 AM.

In the first of two experiments, female mice $2\frac{1}{4}$ months old were selected and divided into three groups (A, B, and C). Those of group A were given alcohol daily by mouth for 22 days according to the above schedule. Food was available at

all times. The mice of group B were not given alcohol, but each day food was removed from their cage at 5:00 PM and returned at 8:00 AM. These animals served, therefore, to demonstrate the effect on resistance of daily fasting for three weeks before infection. The mice of group C were neither alcoholized nor forced to fast, and were used to check normal development under the conditions of the experiment. Twenty-four hours after the last injection was given to the alcoholic mice, each of the animals in the three groups was infected with 900 *Hymenolepis* eggs. Thereafter until autopsy the alcohol injections were discontinued and food was available to all animals. The average daily food intake per mouse, and the average number and percentage development of cysticercoids are given in Table 1. The alcoholic

TABLE 1.—*Comparing the daily food intake and the percentage development of cysticercoids in: (A) alcoholic mice, (B) non-alcoholic fasting mice, and (C) non-alcoholic, non-fasting mice*

Exper. no.	No. mice	Age at infection (months)	Average daily food intake per mouse (grams)	Infecting egg dose per mouse	93-hour cysticercoids		
					Average no.	Range in no.	Percentage development
A. Alcoholic mice							
1	5	3.0	3.2	900	72.8	65–81	8.1
2	6	2.0	3.9	1000	112.8	79–165	11.3
B. Non-alcoholic fasting mice							
1	5	3.0	3.2	900	81.2	68–91	9.0
2	6	2.0	4.0	1000	127.0	91–171	12.7
C. Non-alcoholic—non-fasting mice							
1	5	3.0	5.2	900	32.4	25–41	3.6
2	6	2.0	5.5	1000	52.8	40–69	5.3

mice of group A each consumed an average of about 3.2 grams of food daily as did each of the non-alcoholic fasting mice of group B. This, as expected, was considerably less than the amount (5.2 g) taken by each of the controls in group C. Large checkers of Purina dog chow were used as food. The food for each cage was placed in a wire feeding basket (mesh approximately $\frac{3}{8}$ inch square) suspended into the cage from the top and open only to the outside. By this method the food is protected from contamination and from being scattered and wasted, but at the same time is always available. No attempt was made to recover and calculate the amount that sifted through the feeders into the shavings below, so that the averages given above are in excess of the amount actually consumed. Correlated with the reduced food intake of the mice of groups A and B was an increased percentage development of the parasite. The alcoholic mice (group A) averaged an 8.1 per cent development of cysticercoids and the non-alcoholic fasting mice (group B) averaged 9.0 per cent. These percentages were markedly higher than the 3.6 per cent of the group C controls, which showed typical numbers of cysticercoids for animals of their age infected as above.

In the second experiment, male animals $1\frac{1}{4}$ months old were used. These were divided into three groups and handled as above, the only change in procedure being that each mouse was weighed at the start and finish of the experiment. In this case the alcoholic mice were given 21 daily injections of the drug. The average daily food intake, and the results of infection with 1000 eggs per animal of the three groups of this experiment are likewise tabulated in Table 1. As in the first experiment, the

alcoholic mice of group A and the non-alcoholic fasting mice of group B consumed daily about the same amount of food (3.9 and 4.0 g, respectively), which was much less than the average (5.5 g) taken by each mouse in group C. The alcoholic mice (group A) showed an average weight loss during the experiment of 0.9 g, whereas the non-alcoholic fasting mice (group B) and the controls (group C) showed an average gain of 3.5 gms. As in the first experiment with older mice, the animals of groups A and B with reduced food intake likewise showed a much greater percentage development of cysticercoids than the group C controls, averaging respectively 11.3 and 12.7 per cent, as compared with 5.3 per cent for the mice of group C infected with the same number of eggs. The use of younger mice probably accounts for the increased food consumption and for the higher percentages of development in each group as compared with the first experiment.

The fact that the alcoholic mice and the non-alcoholic fasting mice consumed about the same amount of food and showed a strikingly similar reduction in resistance to infection with *Hymenolepis* indicates that the effect of alcohol on this resistance is indirect by greatly reducing food intake. Since the non-alcoholic fasting mice ate approximately the same amount as the alcoholics and yet gained as much as the controls which ate much more, it would appear that fasting, not the weight factor, was responsible for the reduced resistance.

Despite similar food intake the non-alcoholic fasting mice gained weight during the experiment whereas the alcoholic mice lost weight. This suggests, as mentioned by Myerson, Alexander, and Moore (1942), that in the latter there were subnormal resorptive functions due to intestinal and liver changes produced by alcohol.

2. *The effect of supplemental vitamins in preventing reduced resistance to* Hymenolepis *produced by alcohol.*—Since lowered resistance to *Hymenolepis* was associated with reduced food intake caused by fasting in the above series of experiments, the next step was to search for the particular food factor or factors involved. The first tests were concerned with the influence of vitamins. Two similar experiments were carried out at different times. The mice, 2.3 and 2.5 months old of both sexes, were weighed and divided into six groups (A, B, C, D, E, and F). Three of these (groups A, C, and E) were treated in the same way as the three groups in the experiments of series 1 above. The mice of group A, the alcoholic mice, were given 40 per cent alcohol in 26 daily injections. An initial dose of only 0.2 cc was given, but this was increased gradually from day to day until the maximum dose of 0.4 cc was attained on the sixth day. The animals of group C, the non-alcoholic fasting mice, were denied food each day from 5:00 PM until 8:00 AM. The group E mice were neither alcoholized nor forced to fast. For each of these three groups, there was included a companion group, group B matched with A, D with C, and F with E. Aside from receiving an 8:00 AM daily injection of vitamins, the mice of groups B, D, and F were treated in exactly the same way as those of their corresponding companion groups A, C, and E. Abbott's improved vitamins (Vita-Kaps) were used, each capsule containing the following: vitamin A (5000 U.S.P. units), vitamin C (750 U.S.P. units), vitamin D (500 U.S.P. units), vitamin B_1 (thiamin hydrochloride—3 mg), vitamin B_2 (riboflavin—2.5 mg), vitamin B_6 (pyridoxine hydrochloride—0.5 mg), nicotinamide—20 mg, and pantothenic acid (as calcium pantothenate)—5 mg. The contents of the capsules were removed and dissolved in, or, in the case of the fat-soluble vitamins, suspended in water so that

each mouse of groups B, D, and F received each day in a 0.4-cc dose approximately one-tenth of the contents of one capsule. Twenty-four hours after the last alcohol injection had been given, all of the animals of the six groups were infected with the same number of eggs of *Hymenolepis*. Thereafter until autopsy, the alcohol and the vitamin injections were discontinued and food was made available to all animals. Table 2 shows for the six groups of the two experiments the average daily food intake per mouse, the average weight gain during the experiment per mouse, and the data on infection with *Hymenolepis*.

TABLE 2.—*Showing the daily food intake and the percentage development of cysticercoids in alcoholic mice (A), non-alcoholic fasting mice (C), control mice (E), not given vitamins, and in their companion groups (B, D, and F) given vitamins*

Exper. no.	No. mice	Age of mice at infection (months)	Average daily food intake per mouse (grams)	Average weight gain during experiment per mouse (grams)	Infecting egg dose per mouse	93-hour cysticercoids		
						Average no.	Range in number	Percentage development
A. Alcoholic mice								
1	3	2.5	3.4	0.0	1000	74.0	69–82	7.4
2	3	2.3	4.2	3.8	900	51.3	39–63	5.7
B. Alcoholic mice given vitamins								
1	6	2.5	4.2	0.7	1000	12.7	6–29	1.3
2	3	2.3	3.5	0.5	900	25.3	22–29	2.8
C. Non-alcoholic fasting mice								
1	6	2.5	3.6	1.0	1000	85.7	68–110	8.6
2	3	2.3	3.9	6.6	900	97.3	68–117	10.8
D. Non-alcoholic fasting mice given vitamins								
1	6	2.5	3.3	0.0	1000	34.2	9–59	3.4
2	3	2.3	3.7	6.2	900	47.1	34–70	5.2
E. Control mice								
1	4	2.5	5.5	2.0	1000	34.0	28–41	3.4
2	3	2.3	6.5	6.6	900	24.0	14–38	2.7
F. Control mice given vitamins								
1	6	2.5	4.1	1.5	1000	14.8	7–39	1.5
2	3	2.3	3.8	1.8	900	9.0	6–11	1.0

The results of the two experiments were similar. In both cases, the alcoholic mice of group A, the non-alcoholic fasting mice of group C, and the controls of group E showed somewhat the same ratio of food intake and percentage development as in the previous experiments of series 1. That is, the mice of groups A and C consumed about the same amount of food daily (3.4 and 4.2 g–3.6 and 3.9 g, respectively), which was considerably less than the average consumed by group E mice (5.5 and 6.5 g). Correlated with reduced food intake in the mice of groups A and C were much higher percentages of development of cysticercoids (7.4 and 5.7–8.6 and 10.8, respectively) than in the controls of group E (3.4 and 2.7). This is further evidence, therefore, that here the effect of alcohol on resistance is indirect by interfering with normal food intake.

The most important point of interest in comparing the groups A, C, and E with their vitamin-injected companion groups B, D, and F is in the percentage development of the cysticercoids (Table 2). In every case, the animals that received supplemental vitamins daily (groups B, D, F) showed a markedly lower cysticercoid development than those of companion groups A, C, and E not given vitamins. In the

first experiment, the alcoholic mice given vitamins (group B) showed a percentage development of 1.3, while that of the alcoholic mice not given vitamins (group A) was 7.4. This percentage for the non-alcoholic fasting mice given vitamins (group D) was 3.4, compared with 8.6 for their controls of group C not given vitamins. The same trend was demonstrated by the control mice in that those given vitamins showed a percentage development of 1.5, while those not so treated showed 3.4. In the second experiment, the results were similar but less striking. Another point brought out in the table concerns food intake and weight gain of the mice in the six groups. The majority of vitamin-injected mice, as compared with the mice in companion groups not given vitamins, showed less food consumption and less weight gain. Interpretation of this phenomenon is difficult, however, as the relationship was not consistent throughout. It will be noted that in the second experiment the mice of groups A, C, D, and E gained considerably more weight during the experiment than those of the same groups in experiment one. Since the animals were about the same age in both experiments, there is no apparent explanation for this weight gain difference.

Since the percentage development of cysticercoids in vitamin-injected mice was considerably less than in mice treated in the same way but not given vitamins, these experiments show that the reduction in resistance to *Hymenolepis* produced by alcohol and by forced fasting was prevented with vitamins alone. Avitaminosis, then, was the principal cause of the reduced resistance in both cases. The action of the vitamins as given apparently was not related to stimulation of appetite, as the majority of mice thus treated actually consumed less food daily than those in corresponding groups not given vitamins. Of course, the supplemental vitamins could have increased the efficiency of absorption and assimilation of the food consumed. It is interesting that even the control mice given vitamins (group F) consumed less food and showed greater resistance to infection than those not given vitamins (group E). If additional work substantiates this observation, it would establish a difference in the amount of vitamins needed, and supplied in this food, for normal growth and development and the amount affording unusual protection against this parasite. The variance in vitamin content of diets might be a reason for the differences noted by various workers in the percentage development of the parasite in mice of the same age infected with approximately the same number of eggs.

In conclusion, alcoholic debilitation of mice infected with *Hymenolepis* is due in greatest measure to avitaminosis, which develops when some action(s) of the alcohol interferes with normal food intake, and, undoubtedly, with normal functions of absorption and assimilation. This relationship is not new, since it is well known that avitaminosis often accompanies the abuse of alcohol (Spies and DeWolf, 1933; Strauss, 1934; Blankenhorn and Spies, 1935, 1936; Jolliffe and Colbert, 1936).

SUMMARY

Studies in mice are described, showing the relationship of avitaminosis and alcoholic debilitation in reducing the resistance to *H. nana* var. *fraterna*. Alcoholic mice were shown to consume much less food per day than non-alcoholic controls. When the latter were forced to fast daily during the experiment so that their food intake was about the same as that of the alcoholic mice, they showed after infection a high percentage development of cysticercoids, which was about the same as that of

the alcoholic mice but considerably greater than that of the non-alcoholic, non-fasting controls. · This demonstrated that the effect of the alcohol in reducing resistance to *Hymenolepis* is indirect by interfering with normal food intake. Later tests showed that daily polyvitamin injections during the experiment prevented this greatly increased percentage development in both groups, as well as reducing the percentage in the non-alcoholic, non-fasting controls. This meant that reduced resistance to *Hymenolepis* brought about by alcohol and by forced fasting was due in most part to avitaminosis. Studies are in progress to determine, if possible, the particular vitamin(s) involved.

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THE DEVELOPMENT OF THE MALARIA PARASITE *PLASMODIUM LOPHURAE* IN RED BLOOD CELL SUSPENSIONS IN VITRO

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In earlier studies (Trager 1941, 1943) on conditions affecting the survival and development in vitro of *Plasmodium lophurae,* an avian malaria parasite, it was found that red cell extract, suitable concentrations of glucose, glutathione, calcium pantothenate and serum or plasma, a balanced salt solution having a high potassium content, an optimal density of parasites per cubic millimeter, gentle agitation of the suspension, aeration (but not a high oxygen tension), renewal of the medium, and provision of fresh red blood cells, all favored survival. In the best preparations, small increases in the parasite number occurred during the first few days of incubation (at about 40° C) and living infective parasites were demonstrated after 16 days in vitro. In all these experiments the parasite density ranged from 25,000 to 100,000/cu mm and the concentration of red cells from about 150,000 to 350,000 per cu mm. The total number of red cells was kept low in order to facilitate the observation of the effects of changes in the medium, and indeed effects were observed which probably could not have been noted in the presence of large numbers of normal red cells.

More recently it has been found by others (Ball et al, 1945; Anfinsen et al, 1946; Geiman et al, 1946; McKee et al, 1946) that *Plasmodium knowlesi,* a monkey malaria parasite, will multiply in suspensions of monkey red blood cells maintained in vitro. The medium is a balanced salt solution resembling in composition the inorganic constituents of monkey plasma and containing vitamins, purines, pyrimidines and amino acids. Most of the experiments consisted of observations of the effect of various conditions on the extent of multiplication of the parasites during the first 24-hour period after removal from the monkey, but in one series the parasites were kept growing in vitro for 6 days. Good multiplication even during the first 24-hour period was obtained only in the presence of adequate concentrations of glucose, plasma or serum, and p-aminobenzoic acid. The vessels with the parasitized blood suspensions were rocked (a condition which favored survival of *P. lophurae*) and in addition a stream of 5% CO_2–95% air was passed through them. In these experiments a low parasite density (16,000–25,000 per cu mm) was again found favorable. However, much larger amounts of normal red blood cells were used (1 million or more per cu mm) than in the earlier work with *P. lophurae.*

In vitro studies with *P. lophurae* have now been carried out using combinations of the methods of Trager and of Anfinsen, Ball, Geiman, McKee, and Ormsbee.

METHODS

1. The culture media. The composition and preparation of these is given in Table 1. Solution K was the balanced salt solution of high potassium content previously described (Trager, 1941). Solution BGM was the solution described by Anfinsen et al (1946). For both solutions, vitamins, purines, and pyrimidines

were added in the amounts used for *P. knowlesi* (Anfinsen et al, 1946) except that pyridoxamine was also included and the purines and pyrimidines were added at a 10-fold higher concentration. Water redistilled in pyrex glass was used throughout. Carbon dioxide was bubbled through solution BGM before sterilization to convert the Na_2CO_3 to $NaHCO_3$. All media were sterilized by filtration through a Selas 03 porcelain filter. They were usually tested for sterility and stored before use for one to several days in the refrigerator in pyrex tubes sealed with parafilm.

2. Red cell extract. This was prepared as previously described (Trager, 1943) and was stored in the refrigerator for not over one day before being used.

3. The parasitized red blood cell suspension. Approximately 23 ml of blood was drawn from the neck vein of a normal duck and mixed with 2 ml of heparin solution (27.2 mg of heparin of a potency of 110 units per mg dissolved in 100 ml of 0.85% sodium chloride solution and autoclaved). The blood was centrifuged and 10 to 11 ml of the supernatant plasma drawn off. The cells were resuspended in the remaining plasma. Five ml of blood was then drawn into 0.5 ml of heparin solution from the neck vein of a duckling infected with *P. lophurae* and showing about 30

TABLE 1.—*Composition of the culture media*

Ingredient	Sol. K*	Sol. BGM†	Vitamins‡		Purines and pyrimidines‖	
	gm/l	*gm/l*		*µg/l*		*mg/l*
NaCl	3.040	5.825	Thiamin	1000	Adenine sulfate ...	2.50
KCl	4.100	0.410	Niacin	1000	Guanine hydrochloride	2.50
$MgCl_2 \cdot 6H_2O$	0.400	0.205	Niacinamide	1000	Xanthine ...	2.50
$MgSO_4 \cdot 7H_2O$	0.370	0	Cocarboxylase	400	Uracil ...	2.50
$CaCl_2$	0.160	0.060	d-calcium pantothenate	500	Thymine ...	1.00
$NaH_2PO_4 \cdot H_2O$	0.690	0	Pyridoxine	500	Cytidine ...	1.00
K_2HPO_4	2.610	0	Ribose	500		
$NaHCO_3$	0.170	0	Riboflavin	500		
Na_2CO_3	0	1.480	Choline	500		
Na_2HPO_4	0	0.300	Biotin	0.4		
Glucose	2.38	2.50	p-Aminobenzoic acid ...	100		
Sodium acetate · $3H_2O$	0	0.25	Pyridoxamine	100		
Glycerol	0	0.25	Ascorbic acid§	5000		
Amino acids†	0	0.056				
Glutathione	1.00	0				

* The inorganic ingredients of solutions K and BGM, other than phosphates and carbonates, were kept as stock solutions of 2 or 3 times the final concentration. The phosphates, carbonates and organic ingredients, and, where desired, the vitamins and purines and pyrimidines, were added in the preparation of the final medium.

† A sterile stock solution of amino acids was prepared in the manner of Anfinsen et al 10.6 ml of Stearns' amino acid hydrolysate + 100 mg glycine + 100 mg histidine diluted to 64 ml with water and autoclaved. This was used at 0.2 ml per 100 ml of final medium.

‡ The vitamins (except ascorbic acid) were kept as a sterile stock solution having 100 times the final concentration. One ml was used for 100 ml of final medium.

§ Ascorbic acid was added per 100 ml of the final medium as 1 ml of a freshly prepared solution containing 5 mg in 10 ml of water.

‖ The purines and pyrimidines were added from concentrated stock solutions containing 5 mg per 100 ml of adenine, guanine, xanthine and uracil and 2 mg per 100 ml of thymine and cytidine.

parasites per 100 red cells. The infected ducks were 2 to 4 weeks old and had been inoculated 4 days previously with about 50,000,000 parasites per 100 gm body weight. The parasitized blood was mixed with the concentrated normal blood at the rate of 1 ml of the former to each 4 ml of the latter. The density of red blood cells in the final suspension was determined by hemacytometer count.

4. The cultures. Fifty-ml. Erlenmeyer flasks were provided with 4.5 ml of culture medium. The parasitized red blood cell suspension was then prepared in a suitable quantity and 1.5 ml of it added to each flask. This gave final concentrations of about 50,000 parasites and 1,000,000 red cells per cu mm. The contents of the flasks were mixed and a drop removed from each for the preparation of a stained film. Each flask was then equipped with a sterile rubber stopper bearing gas inlet

and outlet tubes plugged with cotton.   The flasks were placed on a rocking machine in an incubator and were connected to a source of moist 95% air–$5\%CO_2$.   The gas was passed through not over 4 flasks in series.   It was then led out of the incubator and allowed to bubble through water.   The rate of flow was adjusted so that there was approximately one bubble per 1 to 2 seconds at the outlet.   As in the previous work, the rocking machine, driven by an electric motor, went through 18 to 20 complete cycles in a minute. The temperature of the incubator was maintained at 39.5–$40.5°$ C.

5. Observations.   After one and again after 2 days of incubation a drop of material was removed from each flask and used to prepare a thin film which was stained with Giemsa.   The number of parasites per 10,000 red cells was estimated from counts on these smears, as well as on the original smear made just after the preparation of the flask.   At the same time, a differential count of the parasites was made.   For this purpose 50 parasites were counted in successive fields and classified as: Young—very small forms without pigment; Medium—trophozoites, including forms with up to 3 nuclei; Segmenters—forms with 4 or more nuclei; Gametocytes. In the earlier experiments red blood cell counts were made after 2 days of incuba- tion, but since these were not much lower than the original estimated red cell density (based on a count of the parasitized red blood cell suspension), they were omitted in later work.

6. Subcultures.   Flasks were prepared each containing 3.0 ml of culture medium and 1.5 ml of a fresh concentrated normal red blood cell suspension prepared in the same manner as the parasitized red blood cell suspension except that parasitized blood was not added to it.   To each flask was then added 1.5 ml of material from a 2-day old culture.   An initial smear was made, as well as a smear after one and 2 days' incubation.

<center>RESULTS</center>

P. lophurae has regularly multiplied in cultures prepared in the manner described in the preceding section.   Multiplication occurred during the second as well as the first day of culture and similarly during the second as well as the first day of sub- culture.   The relative proportions of the different stages of the parasite changed during the course of the cultures (Table 2).   The extent of multiplication was about the same in solution K plus vitamins, purines, pyrimidines, and red cell extract as in solution BGM plus vitamins, purines, and pyrimidines (Table 3, Exp. 1).   The former medium did not support multiplication if the red cell extract was omitted (Table 3, Exp. 2), but red cell extract had no effect on growth in the complete BGM medium of Anfinsen et al (1946).   The latter was therefore easier to prepare and was used for most of the more recent experiments.

Cultures incubated more than 2 days showed a decrease in parasite number and an increase in degenerate forms.   However, if subculture was effected after 2 days of incubation, the parasites would again multiply over a 2-day period.   In most experiments the parasites were maintained in vitro through one subculture and hence over a period of 4 days.   In one series, employing solution BGM + vitamins, purines, and pyrimidines, subculture was twice repeated until the parasites had been grown in vitro for 8 days.   During the 8-day period they multiplied 170 times, or an average of 1.77 times per day.   However, in this series, as in the much more numer-

TABLE 2.—*Typical changes in the number of parasites and the proportions of the different stages during incubation of* P. lophurae *in vitro*

Preparation*	Parasites per 10,000 red cells at days			Percentage of young (Y), medium (M) and segmenting (S) parasites at days		
	0	1	2	0 Y–M–S	1 Y–M–S	2 Y–M–S
Original culture. Sol. K + V + P + red cell extract	410	1200	1750	34–30–36	30–64–6	16–52–30
Original culture. Sol. BGM + V + P	580	1920	1920	40–42–18	16–84–0	12–74–14
Original culture. Sol. BGM + V + P	670	1830	2700	16–68–16	6–88–6	14–68–18
Original culture. Sol. BGM + V (but without biotin) + P ...	450	920	1380	12–76–12	6–80–14	16–70–14
Subculture from 2-day-old culture. Sol. BGM + V + P	470	1070	1400	4–68–28	16–70–14	10–76–8

* In this and in Table 3, V indicates the vitamins and P the purines and pyrimidines as detailed in Table 1.

ous ones of 4 days' duration, the extent of multiplication tended to be relatively high and low on alternate days (Table 3). This is in keeping with the fact that *P. lophurae* has a 36- to 48-hour, rather than a 24-hour, cycle of development (Terzian, 1941).

Attempts to demonstrate the effects of single substances or even groups of substances in the culture medium have met with considerable difficulty. This was true also in the experiments of Anfinsen et al (1946), and is not surprising in view of the fact that the medium contains rather large amounts of blood cells and plasma, the

TABLE 3.—*Conditions affecting the multiplication of* Plasmodium lophurae *in duck erythrocytes* in vitro

Exp. No.	Flask No.	Medium	Initial parasites per 10,000 red cells	Extent of multiplication† between days				
				0–1	1–2	2–3	3–4	0–4
				Original culture		Sub-culture		
1	1	K + V + P + red cell extract	410	2.9	1.4	4.2	1.2	21.0
	2	BGM + V + P	580	3.3	1.0	2.9	1.0	9.6
2	1	K + V	535	1.1	1.1	..	..	...
	2	K + V + red cell extract	565	1.7	1.2	2.5	1.3	6.7
	3	BGM + V + P	540	1.9	1.6	2.5	1.7	13.0
3	1	BGM + V + P	670	2.7	1.5	2.5	1.7	17.2
	2	BGM + P (no vitamins)	870	1.5	1.5	1.8	1.1	4.5
4	1	BGM + V + P. Blood from normal duck 61 days old	587	1.4	1.7	2.5	1.4	8.3
	2	BGM + V + P. Blood from 61-day-old duck recovered from P. lophurae	660	1.2	1.8	2.1	1.8	8.2
5	1,2	BGM + V (without added biotin) + P. 0.6 mγ biotin per ml	410	1.8	1.9	2.2	1.1	8.3
	3,4	Same but 12 mγ biotin per ml	365	1.8	1.7	2.0	1.2	7.3
	5,6	Same but 120 mγ biotin per ml	420	1.5	2.0	2.2	1.0	6.6
6	1,2	BGM + V + P (without cytidine)	505	1.2	1.7	1.5	1.8	5.5
	3,4	Same + 0.75% plasma protein Fraction V*	545	1.1	2.0	2.5	1.0	5.5
	5,6	Same but 0.75% Fraction II–3*	495	1.6	1.6	1.2	2.0	6.1
	7,8	Same but 0.75% Fraction IV–1,2*	580	1.0	1.2	1.3	1.1	1.7

* The plasma protein fractions used in Exp. 6 were derived from human plasma. They were obtained through the kindness of Dr. L. C. Strong of Harvard Medical School and were prepared under a contract, recommended by the Committee on Medical Research, between Harvard University and the Office of Scientific Research and Development. After hydrolysis by acid they contained the following biotin activities in mγ per gram of fraction: V (Albumin) 57: II–3 (γ-Globulin) 57: IV–1,2 (α-Globulin) 197. Almost all of the biotin activity of Fraction IV–1,2 was accounted for by the fat-soluble material.
† The ratio of the count of parasites per 10,000 red cells on the later day to the count on the earlier day.

inherent variations of which might well be of much greater effect than any small changes in the known portion of the culture medium. Omission of the vitamins from the BGM medium resulted in less multiplication than in control cultures (Table 3, Exp. 3). A wide range of biotin concentrations had no effect on growth (Table 3, Exp. 5) but certain plasma protein fractions rich in a fat-soluble biotin-active material (Trager, 1947) inhibited multiplication (Table 3, Exp. 6).

It is interesting to note that blood from a duck which had recovered from *P. lophurae* infection and which was immune to reinfection supported as good growth of the parasites in vitro as did blood from an uninfected duck of the same age (Table 3, Exp. 4).

DISCUSSION

It must be emphasized that in the cultures of *P. lophurae* described in the present paper, as well as in the cultures of *P. knowlesi* described by Geiman et al (1946), the parasites are actually growing not in the culture medium but inside of living red blood cells. The cultures are comparable not to ordinary cultures of bacteria in non-living media but rather to cultures of viruses or rickettsiae in living tissue cultures. As yet, no malaria parasite has been cultured in a non-living medium of even the most complex composition, let alone in a medium of known composition. The culture methods which have been developed evidently provide optimal conditions for the survival of erythrocytes, within which the malaria parasites grow.

Such cultures are nevertheless valuable for studies on the physiology of malaria parasites. From their use, and from the earlier survival studies with *P. lophurae,* it has been shown that the development of both *P. lophurae* and *P. knowlesi* is hampered by a high oxygen tension (Trager, 1941; Anfinsen et al, 1946) and is favored by glucose, by materials present in serum and by certain of the B vitamins, notably p-aminobenzoic acid for *P. knowlesi* (Anfinsen et al, 1946) and calcium pantothenate for *P. lophurae* (Trager, 1943). It is worthy of note that the discovery of the effect of pantothenate on the survival of *P. lophurae* in vitro led to the synthesis of certain analogs of pantothenic acid (Woolley and Collyer, 1945; Mead et al, 1946) which have since been shown to be effective anti-malarial drugs (Marshall, 1946). These pantothenate analogs constitute the only instance in which a new type of effective anti-malarial drug was found as the result of a rational approach.

Purines and pyrimidines added together have been shown to favor the growth of *P. knowlesi* (Anfinsen et al, 1946). In some experiments with *P. lophurae,* the omission of purines and pyrimidines had a distinctly deleterious effect, but such a result could not be regularly obtained.

SUMMARY

The multiplication of *Plasmodium lophurae* has been obtained in suspensions of duck erythrocytes maintained in vitro. The parasites multiply 2 to 3 times during a 2-day period of cultivation. If they are then subcultured to a fresh suspension of duck erythrocytes in fresh culture medium they continue to multiply for a further 2 days. In this manner they have been kept in vitro for 8 days, at the end of which time they were still multiplying actively.

Omission of vitamins from the culture medium was deleterious to the growth of the parasites, but biotin concentrations ranging from 0.6 to 120 mγ per ml had no effect on the extent of multiplication.

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FIBRILLAR STRUCTURES IN *OPALINA OBTRIGONOIDEA* METCALF[1]

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The systematic position of the opalinid protozoa has been a matter of controversy for many years. Superficial examination places them in the class CILIATA, but from the earliest cytological studies, it has been apparent that they are not typical ciliates. The only structures unequivocally identifiable as nuclei are all similar, and at least two are present in every adult. Hartog (1906) wrote that this nuclear condition requires placing these protozoa among the flagellates, while Neresheimer (1907) thought that sexual reproduction by gametes showed that *Opalina* is more closely related to the PLASMODROMA than to the CILIOPHORA. Most workers, however, have followed Metcalf (1909) in regarding neither of these differences as great enough to outweigh the essential similarity of the locomotor organelles and their associated fibrils in opalinids and typical ciliates. But Gatenby and King (1925) reopened the question by describing in *O. ranarum* an arrangement of locomotor organelles and fibrils which they believed demonstrated flagellate affinities.

The earlier accounts of fibrillar structures in these protozoa are often confusing because it is not possible at present to identify the species studied; it was not until 1923 that Metcalf established the four accepted genera: *Protoopalina, Opalina, Zelleria,* and *Cepedea.* Metcalf (1909, 1923) presented a critical review of the earlier work.

Well before Sharp's (1914) classic paper on the fibrillar structures and morphology of *Diplodinium* (= *Epidinium*) several workers had described fibrillar structures in *Opalina ranarum*. Tönniges (1898) described fibrils connecting the basal granules, attributing to them a contractility which was responsible for the motion of the cilia. Zeller (1877) described the pellicular striations and thought them to be myonemes, as did Konsuloff (1922). Both Schneider (1906) and Konsuloff observed the endoplasmic fibrils and believed that they were form-maintaining organelles. A similar interpretation was given by ten Kate (1927) who also described transverse and longitudinal connectives, similar to those figured by Metcalf (1909), between the basal granules.

In view of the desirability of accumulating considerable diversified information before attempting to settle the question of the true relationships of the opalinids, it seemed advisable to determine the details of the fibrillar structures in an unequivocally identified adult opalinid, using all the standard as well as the more recently developed techniques.

MATERIALS AND METHODS

Infected adult *Rana pipiens* were obtained from commercial sources. After pithing, the rectum was placed in amphibian Ringer's, slit open and washed out. Adult opalinas present were removed to a centrifuge tube in as small a quantity of

solution as possible and fixed immediately in a large excess of fixative. Since opalinas remaining in the Ringer's were alive and motile after 48 hours, it seems unlikely that the fixed specimens could have undergone any changes during their short immersion in the solution. Bouin's, Hollande's, Schaudinn's, Zenker's, Helly's, Flemming's with and without acetic acid, Champy's, 2% osmic acid and saturated corrosive sublimate were used for fixing. Essentially the same pictures resulted with all of them, although the fibrils stained better with hematoxylin after fixatives containing chromium compounds.

Washing, dehydration and clearing were done in bulk in centrifuge tubes, with sufficient eosin added to the absolute alcohol to tinge the specimens red. Cleared specimens were removed individually to small blocks of paraffin which were dropped on the surface of molten paraffin in watch glasses. After standing ten minutes in the oven, the paraffin was solidified by chilling in cold water. The embedded specimens were easily located close to the lower surface of the paraffin. Small blocks, each containing one individual, were cut from the mass and fastened to the object carrier of the microtome in such a fashion that precisely oriented sections could be cut at 10, 5, 3 or 2 micra. Only the thinnest sections are satisfactory for demonstrating the finer fibrils.

Mallory's triple stain and iron hematoxylin destained with 10% aqueous hydrogen peroxide (Kidder, 1933) gave the clearest pictures. Cole and Day's (1940) modification of the Bodian silver method impregnated only the cilia and the pellicle; Horvath's formol-silver method, as given by Pantin (1946), gave clear pictures of the pattern of the ciliary rows. Mallory's gave excellent differentiation of pellicular structures, cilia, basal granules and oblique connectives, but the endoplasmic fibrils could be observed only after prolonged staining with the acid fuchsin component. Hematoxylin, when properly destained, brought out all the fibrillar elements described.

Whole mounts stained with hematoxylin were helpful in working out the details of ciliary pattern, but temporary preparations using the Noland-Osterud stain were very good, the basal granules staining intensely while the oblique fibrils were unstained but brightly refractile.

Living material in which locomotion was stopped with methyl cellulose was observed with both light- and dark-field techniques. It was not possible to observe any finer structure of the cilia by either method, but the ectoplasmic components of the fibrillar complex were more or less clearly visible.

<center>OBSERVATIONS</center>

The body of *Opalina obtrigonoidea* resembles a scalene triangle. The anterior end is slightly convex, as is one lateral edge, while the other is concave. Following Mohr's (1940) terminology, the angle between the anterior end and the convex side is the apex; when it lies on the right, the animal is oriented with its "dorsal" surface uppermost. Except for the posterior portion, the body is dorso-ventrally flattened and the whole animal is somewhat twisted about its longitudinal axis, so that the dorsal surface is convex and the ventral concave.

The body form is constant while individuals are swimming in a liquid or semi-liquid medium. However, in sections of the host rectum, the opalinids are often greatly twisted in conformity with the surface of the epithelium. A passive folding

of the opalinid as it penetrates crevices and a squeezing action of the muscular con-
tractions of the rectal wall are sufficient to account for these shapes; because I have
never observed any spontaneous change of form, I reject the idea of any myonemes
being present.

The pellicle forms the outermost layer of the ectoplasm. It is a thin, apparently
homogeneous structure raised into sharp longitudinal folds which parallel the rows
of cilia. In the middle region of the body where the ciliary rows are farthest apart,
there are five (occasionally six) ridges, separated by narrow v-shaped grooves,
between the ciliary rows. At the anterior end the presence of the intercalated rows
reduces the number of ridges between them to two or three. It is very difficult to
decide whether the cilia emerge from one of these ridges or from a groove between
them, but on the basis of Bodian preparations, I believe the cilia actually emerge in a
wide groove between two adjacent pellicular ridges.

The pellicle is bounded internally by a deeply staining membrane which marks the
boundary between it and the remainder of the ectoplasm.

Just internal to this membrane a pair of fibrils forms the falx which runs along
the antero-ventral surface. In favorable sections, these fibrils are seen to be con-
nected by stout fibrils, and to give off a similar fibril to the first basal granule of
each ciliary row. As the falcular fibrils pass around the left anterior corner of the
body, they fuse to form a single, more delicate fibril which continues some distance
posteriorly along the left edge, giving rise to ciliary rows (Fig. 3). Stout cilia,
without basal granules and approximately twice as thick as the remainder of the
cilia, spring directly from the falx; I have called them the falcular cilia. The falcular
fibrils apparently correspond to the Wachstumzone of van Overbeek de Meyer
(1929). Between them there is a furrow in the pellicle which Hara (see Mohr,
1940) interpreted as a cytostome, but there is no satisfactory evidence for such an
interpretation in my material. The furrow may, however, represent the vestige of
a former cytostome.

The ciliary rows of the dorsal surface are sigmoid while those of the ventral
surface are approximately rectilinear. About one-half of the total number of rows
pass completely around the body in a spiral fashion (Fig. 1), while the remainder
extend only part of the distance from the anterior end to one edge. These latter
constitute the intercalated rows and probably represent stages in the growth of new
ciliary rows.

The spherical basal granules are situated a short distance internal to the pellicle.
They lie so close together in longitudinal rows that it is doubtful that any longitudinal
connections between them can be demonstrated with light microscopy. Even when
the basal granules are well separated at the posterior ends of intercalated rows, no
longitudinal fibrils are visible. However, each granule is connected with a granule
in the rows on each side of it by a distinct oblique fibril which runs approximately at
right angles to a tangent to the ciliary row. In cross-sections, these fibrils are seen
to be attached to the internal end of the basal granules.

Extending internally from the inner end of the basal granules are much stouter
fibrils, almost as thick as the cilia. Not every basal granule shows one of these
fibrils; they are least common on the basal granules of the edge of the body.
Each fibril passes through the remainder of the ectoplasm and continues into the
endoplasm without abrupt change in size or direction. In its course it may be

more or less curved.   Most of them continue to the opposite surface of the body where they end in another basal granule.   Accordingly, I have used ten Kate's term dorso-ventral fibril for them.   Many of them branch and anastomose in their course through the endoplasm.   Since they do not pass directly across the body in any direction, it is not possible to trace all the fibrils in any given section to their basal granules.   Especially in transverse sections, many of them appear as granules which upon careful focussing can be seen actually to be sections of a fibril.   Such granules constitute but a small portion of the total number of granules in the endoplasm.

Some of the fibrils appear to end in an endoplasmic spherule (Fig. 6) but careful study shows that another fibril always emerges from the opposite side of the spherule. I believe that these fibrils are continuous and merely in close apposition to a spherule, so that it is impossible to trace their continuity due to the densely staining character of the spherule.

Others of these fibrils appear to end on the nuclear membrane without showing any further subdivision to form a peri-nuclear network.   Many sections show a nucleus in some stage of mitosis, and it is obvious from these that the dorso-ventral fibrils which end on the nuclear membrane do not play any regular rôle in nuclear division, since none of these nuclei shows any polar granule or fibril.   Careful study shows that some of these fibrils only touch the nuclear membrane and pass along it, thereby passing out of the section.   Others, however, actually end where they touch the membrane since no prolongations of the fibrils beyond this point can be traced.

On superficial examination, the endoplasm appears highly reticular, but careful study shows that this is due to two conditions: in the vicinity of the endoplasmic spherules the cytoplasmic granules are more numerous and when not properly de-stained appear to form fibrils radiating between spherules; unless the sections are properly destained it is impossible to distinguish one granule from another so that a reticular appearance results.   Both these difficulties are avoided in Mallory-stained preparations in which the granules are blue and the spherules red.   In such sections the endoplasm is rather coarsely granular while the ectoplasm is finely granular above the basal granules and shows indistinct alveoli internal to them.

Hara's (1937) description of *O. obtrigonoidea japonica* agrees essentially with the above account except that the pellicular grooves (= "pellicular lines") are more numerous (10–20 between ciliary lines) and no oblique fibrils are figured.   Follow-ing ten Kate (1927), Hara distinguished the ectoplasmic portion (= "inner fiber") of the dorso-ventral fibril from its endoplasmic portion, which he described as form-ing a network.   Since these fibrils do branch and anastomose, they probably form a network in my material also, but I have not been able to get satisfactory prepara-tions of sufficient thickness to show it as clearly as Hara did.   As already stated, I have not been able to see the longitudinal fibrils reported by him.

INTERPRETATIONS

Attempting to ascribe a function to any of the fibrils is difficult and probably futile without more experimental evidence.   I do not believe that the dorso-ventral fibrils are morphonemes.   They are often suggestive of slack lines, while they should be taut if they maintain body form, although it is not impossible that their apparent slackness is a fixation effect.   In addition, they occur at the posterior end

of the body which is circular in cross-section and where presumably no morphonemes would be necessary. Needham and Needham (1926) in microinjection experiments observed that the pellicle was tough, as did van Overbeek de Meyer (1929) during micro-dissections. Moreover, Needham and Needham also observed that indicators injected into the endoplasm failed to spread and they believed that this failure could be explained by a gelatinous consistency of the endoplasm, or by the "recent observations of Gatenby and King" which "suggest that Opalina is not a ciliate at all, but a colony of flagellates, in which case it is not impossible that between the nuclei there may be membranes limiting the spread of the dye." A gelatinous consistency of the endoplasm is further supported by van Overbeek de Meyer's observation that the endoplasm does not flow out through an incision, and by the absence of Brownian movement of any of the endoplasmic inclusions in living material. Some workers would ascribe such a consistency to the fibrils present, but I do not believe that they are sufficiently numerous to warrant such an interpretation. If the endoplasm is gelatinous, it is difficult to see why any special fibrils would be necessary to maintain body shape. If any structures are actually required for that purpose, I believe that the pellicle and its ridges are the most likely ones, with the latter functioning much like the corrugations in corrugated cardboard. If the endoplasm is gelatinous, it also hardly seems necessary for any of the fibrils to function in holding the nuclei in one place, as suggested by ten Kate (1927), provided it is even considered necessary for the nuclei to remain fixed.

On the other hand, if the dorso-ventral fibrils are assumed to be conductile in function, it is difficult to reconcile their distribution with the beautifully regular ciliary action. The oblique fibrils fit in well with such a function, however.

No well-differentiated motorium is present, although it is not unlikely that the falcular fibrils represent its homologue. The beginning of each ciliary wave can be seen to start approximately at the apex and travel obliquely across the body. It is possible that the impulse is generated at the right end of the falx, travels from it to the first basal granule of each ciliary row, and then from granule to granule along the oblique fibrils. This interpretation is perhaps also supported by the shape of the advancing wave of ciliary action, which conforms roughly to the distribution of the oblique fibrils.

SUMMARY

1. Intercalated ciliary rows are present in addition to complete rows which extend spirally around the body, being curved on the dorsal and nearly straight on the ventral surface.

2. A pair of fibrils courses along the anterior end just beneath the pellicle, giving rise to the ciliary rows and to stout cilia. These falcular fibrils perhaps represent the homologue of a neuromotorium.

3. Each basal granule of a row is connected with those in the two adjacent rows by oblique fibrils, but no longitudinal fibrils could be demonstrated.

4. Stout fibrils extend through the endoplasm from some of the basal granules, either attaching to others on the opposite side or ending on the nuclear membrane.

5. The possible functions of the observed differentiations are discussed.

6. No evidence is furnished by the fibrillar structures of *Opalina obtrigonoidea* to support the opinion that it is closely related to the PLASMODROMA; conversely,

the presence of typical basal granules and connectives indicates affinities with the CILIOPHORA. Fibrils similar to the dorso-ventral fibrils of *Opalina* have been described in some of the more primitive holotrichs.

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EXPLANATION OF PLATE

Abbreviations: A—apex; b.g.—basal granule; c.—cilium; d-v.f.—dorso-ventral fibril; e.s.—endoplasmic spherule; f.c.—falcular cilium; f.f.—falcular fibril; g.—groove between falcular fibrils; i.r.—intercalated row; n.—nucleus; p.g.—pellicular groove; p.r.—pellicular ridge; t.f.—oblique fibril.

All figures except Fig. 1 are from hematoxylin-peroxide preparations, × 1440.

FIG. 1. Diagram of the pattern of the ciliary rows; approximately one-half of the total number present are shown. Continuous lines—rows on the dorsal surface; broken lines—rows on the ventral surface. Dorsal aspect. Formol-silver preparation.

FIG. 2. Surface view of a portion of the anterior region showing details of the basal granules and their associated fibrils.

FIG. 3. Lateral view of the posterior end of the fused falcular fibrils. Semi-diagrammatic; body cilia omitted.

FIG. 4. Longitudinal section through the anterior end showing the two falcular fibrils and their connections.

FIG. 5. Transverse section through the middle region of the body.

FIG. 6. Longitudinal section through the middle region of the body. Only a portion of the entire section is shown. Cilia omitted.
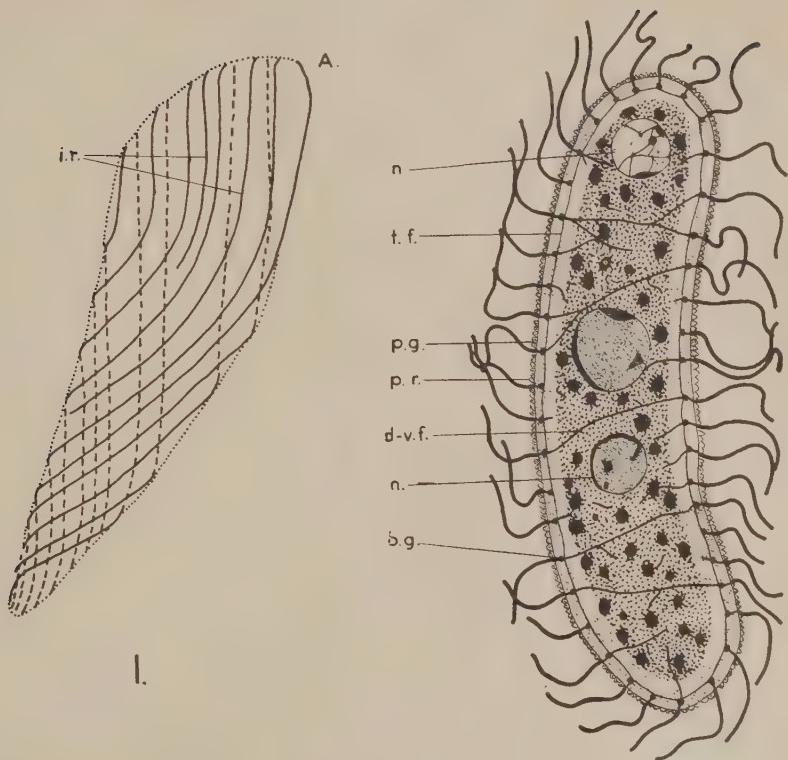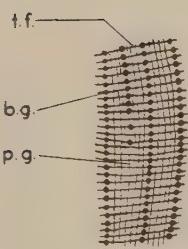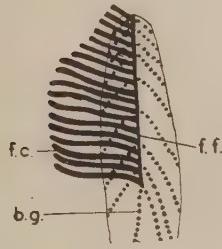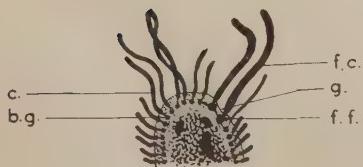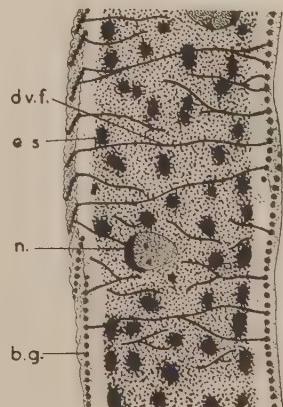
IN VIVO EFFECT OF METACHLORIDINE ON CHRONIC TRICHOMONIASIS IN PIGEONS

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Metachloridine (2-metanilamido-5-chloropyrimidine) has proven very effective in certain aspects of *Plasmodium cathemerium* (Hughes and Brackett, 1946; Gingrich, Schoch, and Taylor, 1946) and *P. gallinaceum* (Brackett and Waletzky, 1946) infections in canaries and chickens, respectively. It has been used unsuccessfully in infections with *Trypanosoma equiperdum, T. cruzi, Leishmania donovani, Schistosoma mansoni,* and *Eimeria tenella* (Brackett and Waletzky, 1946). Because of its effectiveness in avian malaria it seemed worth trying against other pathogenic protozoa. *Trichomonas gallinae,* the cause of pigeon canker, was selected.

Six chronically infected white Carneau pigeons[1] were divided into two birds as controls, with four receiving the metachloridine.[2] Observations on weight and water consumption were made daily on all six birds for ten days prior to treatment. The drug was administered in the drinking water of the four experimental birds in the amount of 150 mg per kilogram of body weight per day for a period of seven days.

All six birds were positive for *T. gallinae* at the end of the week's exposure to the drug. They were then carried nine days with no drugging, at the end of which time they were found to be still swarming with *T. gallinae.* All examinations of the birds were made on fluid removed from the pharyngeal portion of the oral cavity.

It is concluded that metachloridine administered in the drinking water at the rate of 150 mg per kg. of body weight per day to pigeons chronically infected with *Trichomonas gallinae* in the upper digestive tract has no appreciable effect on the protozoan.

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TRICHOSTRONGYLUS INFECTION IN MAN AND DOMESTIC ANIMALS IN JAVA

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INTRODUCTION

Although competent investigators like Darling, Barber and Hacker (1920) Schüffner (1912), Brug (1921) and many others have studied ancylostomiasis and related intestinal helminthic infections in the Malayan Archipelago, the presence of trichostrongylosis in man escaped attention until 1939. This suggests how easily this infection can be overlooked. The worms are so delicate that they are not discovered by routine post-mortem technics. However, when standard helminthological technics are carried out, i.e., scrapings made of the duodenal and jejunal mucosa, mixed with water and stirred, the suspension allowed to settle out and the sediment then examined under the binocular microscope, adult *Trichostrongylus* are found side-by-side with the still smaller adult *Strongyloides*.

POST-MORTEM EXAMINATIONS

With the technic described above the bodies of 119 Indonesians and 32 Chinese were examined and *Trichostrongylus* was found in 49 Indonesians and 6 Chinese. The Indonesians included 84 men and 35 women, with 31 and 18 infections, respectively. In 5 autopsies the whole intestinal tract was examined by dividing it into a number of segments and examining each segment separately. A large majority of the worms were recovered from the duodenum and the upper part of the jejunum, although occasionally parasites were present in the stomach or the lower levels of the intestine.

Two species of *Trichostrongylus* inhabit man in Java, namely *T. colubriformis* (Giles, 1892) Ransom, 1911 and *T. axei* (Cobbold, 1897) Railliet and Henry, 1909.

The commoner species is *T. colubriformis*. It was the only one found in the 6 positive Chinese cases and was present in every positive Indonesian. In 11 Indonesians there was a mixed infection with *T. axei*.

The frequency of *Trichostrongylus* infections does not diminish with increasing age of the individuals examined (Table 1).

TABLE 1.—*Number of bodies examined and number positive for* Trichostrongylus, *by age groups*

Estimated age	Number of bodies examined	Positives
5–19 yrs.	8	3
20–29	20	8
30–39	31	11
40–49	28	10
50 and older	32	17

In a majority of human infections in Batavia the number of worms is small. In the series mentioned above and observed during the years 1939–1941 the greatest number found in one patient was 73 for *T. colubriformis* and 8 for *T. axei*. But it is

certain that even when following the described technic, several, probably a large percentage of the worms present, are missed.   In later years one particularly heavy infection was observed.   In a post-mortem of a mentally subnormal Indonesian patient at least 5,000 *Trichostrongylus* together with hundreds of hookworms were collected.   The number of females discovered always considerably exceeded the number of males, a phenomenon mentioned by Looss (1895).

With the same technic the intestines of goats, sheep, cattle, water-buffaloes, pigs, dogs, cats, rabbits, monkeys, rats, chickens, ducks, pigeons and a few other species of birds were examined.   A high incidence with heavy infections of *T. colubriformis* was found in goats and sheep and a low incidence with light infections in monkeys and rabbits.   In goats and sheep the worms lived mostly in the duodenum and the upper part of the jejunum and sometimes in the fourth stomach.   In monkeys and rabbits they were present in the upper part of the small intestine.   A high incidence with heavy infection of *T. axei* was found in goats, sheep and cattle, always in the fourth stomach.   The other animal species examined were negative.

Detailed information on the morphology of the adult worms and their larval stages has been presented by several workers, including the writer (1941), and needs no special consideration in this communication.

EXPERIMENTAL INFECTIONS

Infection of a human volunteer with T. colubriformis.—A few hundred female *T. colubriformis* from the intestine of a goat were collected in a little water in a large-sized watchglass and left in the incubator at 37° C.   The worms deposited most of their eggs.   A culture was set up with these eggs on worm-free sand to which a small quantity of an extract of goat's feces was added.   Even in this medium, which gave better results than other media tried, the percentage of larvae developing was comparatively small.   Apparently many eggs expelled from the uterus were inferior in quality and did not complete embryonation.   Since controls remained negative, the resulting culture of *T. colubriformis* was considered as pure.

A human volunteer, free from hookworm- or *Trichostrongylus*-infection swallowed 60 infective *T. colubriformis* larvae mixed with a little bread and water.   The first *Trichostrongylus* eggs, very few in number, were found in his stools 25 days later by the Clayton Lane concentration method.   The infection was very light.   For a period of two months the stools were examined regularly but the maximum of eggs discovered during this time was only 11 in about 12 grams of feces.   Seven weeks after the infection the percentage of eosinophils in the volunteer's blood rose from 1.5% to 10%.   Five weeks later it had come down to 4.5%.   Thereupon the volunteer reinoculated himself with 190 infective *T. colubriformis* larvae and 21 days after re-exposure there was a sudden increase to 80 in the number of eggs present in about 12 grams of stool.   During the following weeks this number rose to 100–150.   There was a rise of eosinophils to 7% a month and a half after the superinfection.   Two months later, however, it had returned to the original 1.5%.   The volunteer remained healthy during this period.   There were no changes in his hemoglobin or in the number of erythrocytes and leucocytes.

A second human volunteer, inoculated with 140 infective *Trichostrongylus* larvae obtained from volunteer No. 1, showed eggs in his stools for the first time 21 days later.   This seems to be the normal period elapsing between exposure and the first

appearance of the eggs in the stools of man. In the very light original infection of volunteer No. 1 their presence may have been overlooked between the 21st and the 25th day.

Infection of worm-free, milk-fed young goats with T. colubriformis.—A young goat was separated from its mother $1\frac{1}{2}$ days after birth and kept worm-free for $2\frac{1}{2}$ months. It was then exposed to 80 infective *Trichostrongylus* larvae obtained from volunteer No. 1, by introducing the larvae in its mouth with a pipette. Its stools were at that time negative. Twenty-one days after the infection the first eggs appeared. Between one month and $3\frac{1}{2}$ months after the first infection this goat was reinfected 25 times with a total of about 11,000 larvae of *T. colubriformis,* obtained either from the stools of volunteer No. 1 or from the goat itself. Yet the number of eggs in the goat's stools did not show the enormous rise which might be expected. When the animal was killed 4 months after the first inoculation 614 specimens of *T. colubriformis* and no other worms were found in its intestine. The infection appears to be more or less self-limiting. In a second experiment, using a litter of two goats, one was infected by mouth on 8 successive days with about 1,000 infective *T. colubriformis* larvae obtained from human volunteer No. 1. The other goat received the same food and served as a control. Twenty-nine days after the first exposure both animals were killed. The control goat was negative. Examination of the infected goat showed 680 *T. colubriformis,* 315 males and 365 females, and no other worms.

From these experiments it may be concluded that volunteer No. 1 had a pure infection with *T. colubriformis.* No biological differences have been discovered between *T. colubriformis* developing in man and in goats. Man is just as easily infected with *Trichostrongylus* from the goat as the goat with *Trichostrongylus* from man; eggs appeared in the stools 21 days after the infection in both cases. Under natural conditions in Java, goats and sheep serve as reservoir hosts but the goat is the more important of the two, being much more common and living in very close association with man.

EXPOSURE OF HUMAN VOLUNTEERS TO *T. axei*

Three times an attempt was made to infect a human volunteer with the infective third-stage larvae of *T. axei* obtained from goats. One hundred twenty larvae were given each time. The results were consistently negative. This volunteer became readily infected with *T. colubriformis,* the eggs appearing in his stools on the 21st day, which suggests that man is a relatively refractive host for *T. axei.* In nature the chances of infection with larvae of this species are at least equal to those with *T. colubriformis* larvae, since *T. axei* lives in enormous numbers in sheep, goats, and cattle. The incidence of human infections with *T. axei* is much lower, however, than with *T. colubriformis* and all *T. axei* infections in man are very light.

ORAL VERSUS CUTANEOUS INFECTION WITH *T. colubriformis*

One hundred fifty infective third-stage larvae of *T. colubriformis* were placed on the wet skin of the forearm of a human volunteer. This failed to produce infection and no special signs of irritation of the skin were observed. *Trichostrongylus* larvae are even more resistant than hookworm larvae and every area accessible to ruminants should be considered as infested and hence a source of infection for man. If cu-

taneous infections were possible in man, human infections in nature might be expected to be much heavier than those actually observed. The only portal of entry in man is apparently the mouth.

SYMPTOMS OF TRICHOSTRONGYLIASIS

No symptoms of sickness occurred in the above-described human infections. A transient eosinophilia developed with a maximum of 10%. But these experimental infections were all very light. Heavy infections in animals are frequently pathogenic and it seems unlikely that human infections with thousands of *Trichostrongylus* are harmless.

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SUMMARY

Two species of *Trichostrongylus* occur as human parasites in Java, namely *T. colubriformis* and *T. axei*. *T. colubriformis* is the commoner, *T. axei* the rarer species. The number of worms present per individual is usually very small, but more than 5,000 worms were once collected in a post-mortem of a mentally deficient Indonesian.

Goats and sheep serve as reservoir hosts for *T. colubriformis*, goats, sheep and cattle for *T. axei*.

Experimental oral infections of man were successful with *T. colubriformis* larvae cultured from eggs discharged in goats' feces, but similar experiments with *T. axei* larvae were negative. Young worm-free, milk-fed goats were easily infected with *T. colubriformis* larvae from human sources. In man as well as in goats 21 days elapsed between exposure and the first appearance of eggs in the stools. A cutaneous infection experiment in man with *T. colubriformis* produced negative results.

Light infections apparently do not produce clinical symptoms in man. A transient eosinophilia develops which lasts only a few months. Nevertheless it seems improbable that the heavier human infections are harmless.

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NOTES ON FILARIASIS IN LIBERIA

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For many years the central tropical belt of Africa has been recognized as being a region important for various indigenous filarioid infections (Strong et al, 1930; Craig and Faust, 1943). According to Strong, *Filaria (Wuchereria) bancrofti* was found in one instance and *Loa loa* in one instance during examinations of 105 natives of Gbanga in central Liberia. Elephantiasis, particularly in the lower extremities and scrotum, was also reported by him as occurring commonly in Liberia, particularly among the adult Krus. That there is considerable literature on the distribution of these infections in the eastern part of the continent is evident from the review by Hawking (1943), but references to their occurrence in West Africa are scanty.

The present paper describes our recent observations on filariasis in one corner of this highly parasitized part of the world, the Republic of Liberia. During the course of rather extensive surveys of malaria in Liberian civilians in the late spring and summer of 1943, the 14th Malaria Survey Unit of the U. S. Army became interested in acquiring specific information on the incidence of filariasis in the native population and was concerned with the possibility of the transmission of this and other insect-borne diseases to American troops stationed in the country. Numerous cases of microfilarial infections were discovered in native communities immediately adjacent to the Army Post of Roberts Field, which is located about 40 miles southeast of Monrovia. A floating native population had been drawn from all parts of the country to the Roberts Field district on account of the opportunities for employment offered there by the American Army and the Firestone Plantation.

Suspicion that filariasis in the region is mosquito-borne was aroused by the finding of nematode worms in female *Anopheles gambiae,* which were being dissected in the search for malarial sporozoites. A number of specimens of *A. gambiae* infested with nematode larvae was encountered during this work. The worms were imbedded in the thoracic and head muscles, and in at least one case were lying in the proboscis at the time of dissection. They measured 1.5 to 2 mm in length and about 30μ in width, the size of infective *Wuchereria bancrofti* larvae. Since it is well known that *A. gambiae* is an important vector of these parasites, there is good reason to believe that they were *W. bancrofti.* This opinion was confirmed by members of the Firestone Plantation Medical Staff. During each of the months of June and July three infestations of these nematodes in female *A. gambiae* were found. None was seen in the 19 dissections of May, the 141 dissections of August, or the 119 dissections of September. The six filarial infections in a total of 649 mosquitoes represents an incidence of 0.9%. By way of comparison, it can be noted that the malarial infection rate (oöcysts and sporozoites) in the same mosquitoes was 3.4%. Since no other species of mosquitoes were dissected, except a few *A. hancocki,* we have no definite information as to other mosquito vectors of the helminths. However, well over 90% of all the mosquitoes collected at Roberts Field were *A. gambiae,* and, according to the observations of our entomologists, this species was the principal attacker of man in that district.

During our routine inspections and searches for mosquitoes in the native towns and villages, many cases of elephantiasis were noted. In Monrovia, the disease seemed to affect the legs and ankles predominantly. In the vicinity of Roberts Field, enlargement of the scrotum in men and the breasts in women were the commonest types of elephantiasis. More men than women were seen to have enlargement of organs. Few cases of extreme hypertrophy of members were observed, possibly because an appreciable amount of surgery had been performed in the preceding eight months by Firestone and U. S. Army surgeons. Scrota the size of large grapefruits were not uncommon. In the scrotal cases, venereal disease complications were frequent. Natives in their twenties and thirties seemed to be the afflicted age group.

The technique used for the finding and the identification of microfilariae in all the surveys mentioned in this paper was the thick blood film technique. Several drops of blood were drawn from the finger onto a glass slide, spread until the blood covered a surface about the size of a dime, dried and stained for about 40 minutes in dilute Giemsa. A few fresh samples were examined before the blood dried. The Giemsa stain was very satisfactory for demonstrating the structural features of the microfilariae. It did not, however, bring out the sheath of *Wuchereria bancrofti* very well. With the thick film technique, from one to 30 or 40 microfilariae were demonstrated in individual preparations. These preparations were particularly useful in making simultaneous surveys for plasmodia and microfilariae.[2]

The first case of microfilaremia which we discovered was in a small boy, less than five years of age, living in "December's" Village. This smear was taken in the daytime on June 24. The microfilariae were *Acanthocheilonema perstans*.

Seven other cases of microfilaremia, without obvious physical manifestations, were found in adult male natives employed as laborers on the Roberts Field Post, on June 5 to June 8. The larvae in all of these cases were *Wuchereria bancrofti*. In one man the worms were detected in a daytime smear. The other six positive smears happened to be taken after dark.

Another group of male native laborers gave an appreciable daytime incidence of microfilaremia. On August 14, thick films (prepared primarily for a survey of trypanosomiasis) were taken in the late afternoon, between 3:30 and 5:00 PM, from 166 workers as they were going through the "rice line." The men ranged in age from 16 to 40 years. Seven infections (4.2%) with *W. bancrofti* and one infection (0.6%) with *A. perstans* were found. The appearance of *W. bancrofti* larvae in the peripheral blood in an appreciable proportion of the group in the daytime, and in hard-working, apparently healthy men was of some interest to us. Several of these individuals, including the one positive for *A. perstans,* were later examined at night and were found to be positive.

The number of Liberian natives whose blood samples were taken in essentially daytime surveys was 2134. The sixteen cases of microfilaremia mentioned above represent an incidence of 0.75%.

As a consequence of the casual finding of *W. bancrofti* microfilariae in the daytime, several night-time surveys were planned in order that a more accurate estimate of the prevalence of filariasis in this region might be determined. The first group to be studied was a community of women living in the Health Center Villages of "Shangri-La," "Paradise," and "Idlewylde." They were, for the most part, young

women between the ages of fifteen and thirty-five, of average or superior health and vigor. Two of them had elephantiasis of the breasts. Unfortunately, they were not available for blood examination at the time of this survey. The standard thick blood films were taken from 297 of these people on August 3 between 9 : 30 and 11 : 00 PM. The total infection rate was 8.8%. The results of this survey are shown in Table 2. All the microfilariae were *W. bancrofti.*

On August 13 a similar nocturnal study was made of 224 male native laborers (including a few boys) living in Village "E," a settlement also near the Roberts Field Post. There was a greater age spread among this group than among the Health Center group. Two hundred and twenty-four smears were taken, of which seven were positive for *W. bancrofti* and one was positive for *A. perstans.* The filarial infection incidence was 3.6%. See Table 2. Why this rate should be so much lower than it was in the apparently comparable Health Center group is puzzling. Possibly an age factor, a sex factor, or geographical factors relating to the origin of these people in different sections of the Republic are involved.

Another night survey, between 9 : 30 and 11 : 00 PM was made on August 30 of the inhabitants of the native village of "Freetown" where families—men, women and children—were living together. This village is also near the Roberts Field Post. In this survey an attempt was made to break down the population into age groups. A total infection incidence of 13.0% was found. The results are shown in Table 1. All of the microfilariae were *W. bancrofti.*

TABLE 1

Age, years	No. of smears examined	Filarial infection	
		Number	Per cent
Less than 5	3	0	0.0
6–12	12	2	16.7
13–20	104	14	13.5
Over 20	74	9	12.2
	193	25	13.0

The small number of cases in the lower age group, up to 12 years, gives little information about the infection rate in the children. Likewise, our data do not give statistically significant proof of sex difference : twenty-three of the 165 males examined were infected (13.9%), while two of the 28 women were positive (7.1%). It is of some interest to note that in this population, the adolescent group (13–20 years) appears to have developed a transmissible infectivity (13.5%) which is as great as in the older people (12.2%). This would indicate that an infection is built up rather early in life and is maintained, either continuously or by reinfection, for years.

As a precautionary measure for the health of the neighboring military personnel, indicated by the detection on August 3 of 8.8% of microfilaremia in the women living in the Health Center Villages, the infected individuals were urged to leave the area. Some of them certainly did move out, and other tenants took their places. On the night of September 10 a repeat survey was made of the residents of the three Health Center Villages. The results are shown in Table 2. All the microfilariae were *W. bancrofti.*

Because of the rotation of tenants in the villages between August 3 and September 10, it is not known exactly how many old residents were examined for the second

time and how many newcomers were tested for the first time on September 10. However, it is clear that about 90% of the population remained constant and nine of the positives of the August 3 survey were re-examined and were still positive on September 10. If these nine cases are deducted from the September 10 totals, then 16 out of 232, or 6.9%, were infections which had been missed on the first examination, or, possibly, had been picked up in new arrivals.

In view of the prevalence of both symptomatic and "asymptomatic" filaremia in this territory, it seemed advisable to check the military personnel to see if any microfilariae could be found in them. Thick blood films were collected at night from 431 American soldiers (about 95% of those who had been in residence at the Post for one year or longer). No microfilariae were found.

A résumé of the night-time surveys which were made among the native Liberian population is given in Table 2.

TABLE 2

Native village	Date examined	Number examined	Filarial number	Infections, per cent
Shangri-La (1)	Aug. 3	148	9	6.1
Shangri-La (2)	Sept. 10	130	14	10.8
Paradise (1)	Aug. 3	116	11	9.5
Paradise (2)	Sept. 10	100	9	9.0
Idlewylde (1)	Aug. 3	33	6	18.2
Idlewylde (2)	Sept. 10	11	2	18.2
Village "E"	Aug. 13	224	8*	3.6
Freetown	Aug. 30	193	25	13.0
Total		955	84	8.8

* One case of *Acanthocheilonema perstans* was found here. All others were *Wuchereria bancrofti.*

SUMMARY

1. Larval filariids were found by dissection in six out of 649 (0.9%) female *Anopheles gambiae* collected in the vicinity of Roberts Field, Liberia.

2. Numerous cases of elephantiasis, notably scrotal enlargements, were observed abong the Liberian natives.

3. The thick blood film technique as used in malaria diagnostic examinations was employed in the microfilarial surveys described in this paper.

4. In blood smears taken in the daytime, fourteen cases of infection with *Wuchereria bancrofti* and two cases of *Acanthocheilonema perstans* were detected in 2134 Liberian natives, a microfilarial rate of 0.75%.

5. Four nocturnal surveys for microfilariae were made of the residents of five native villages. These surveys showed about 8.8% of the population to be positive for larval worms. All cases were *W. bancrofti* except one infection with *A. perstans*.

6. A breakdown of the microfilarial infection by age groups, in one of the villages, indicated that infection is acquired during the early years of life and continues into adulthood.

7. The cases of microfilaremia which were found in the surveys mentioned above were "symptomless," with one or two possible exceptions.

8. A blood survey was made of 431 American soldiers who had been stationed in Liberia for a year or longer. No microfilariae were found in any of this personnel.

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A FURTHER ACCOUNT OF TSUTSUGAMUSHI FEVER AT SANSAPOR, DUTCH NEW GUINEA

JAMES T. GRIFFITHS, JR.[1,2]

In a previous paper (Griffiths, 1945) the author described the course of a tsutsugamushi (scrub typhus) epidemic as it occurred among American troops after their landings at Sansapor on the Vogelkop peninsula of Dutch New Guinea. The outbreak occurred during August and September of 1944 with occasional cases being recorded until the time that the base was finally abandoned in 1945. Blake et al (1945), Kohls et al (1945), and Philip and Kohls (1945) reported the successful propagation of strains of *Rickettsia orientalis* from chiggers of the species *Trombicula akamushi* (syn. *T. fletcheri*) from the Dobodura area of New Guinea and *Trombicula deliensis* (*T. walchi*) from Bat Island. Philip and Kohls (1945) also obtained strains of the causative organism from two rats (*Rattus concolor browni*) on Bat Island. Blake et al (1945) stated that *Rickettsia orientalis* had been demonstrated in one specimen of *Rattus mordax* at Dobodura. These findings definitely established the importance of the rat-mite relationship in the study of tsutsugamushi fever in New Guinea. Therefore, it appeared that a study of the rats and mites found in the various environments at Sansapor might afford valuable information for the better understanding of the epidemiology of tsutsugamushi fever. The present account deals with the rats and mites taken in the Sansapor area and also with some peculiarities of local epidemiology.

The environments found on the mainland at Sansapor could be roughly divided into three main types (Griffiths, 1945): climax rain forest, abandoned native village and garden areas, and beach and forest margin. Although the two latter types were definitely proven to be sources of tsutsugamushi fever, no evidence to implicate rain forest was found.

Rat traps were placed in both cleared and uncleared sectors of the various associations noted above. Thus, rats with their attached mite populations could be collected and any differences between environmental areas studied. The traps were made by removing one end from a 55-gallon steel oil drum. The drum was buried in the ground to a point within a few inches of the top. Wooden paddles were balanced about the sides, and bait (usually "C" rations) was placed at the inside end of the paddle. When the rat approached the bait, the paddle was so balanced as to fall with the rat into the barrel. These traps were baited in the evening and rat collections were made the following morning. The mites were removed from the rats within 30 to 45 minutes after the rat had been killed. They were preserved in alcohol for later identification.

It was possible to preserve only a sample of the rats captured. These were sent to Mr. Ellis Troughton at the Australian Museum in Sydney, Australia, for identification. Positive identification was sometimes impossible due to the fact that the New Guinea species have not been thoroughly classified. According to Mr. Troughton, the majority of the rats represented the "Vogelkop race of the small and widely distributed rat of the *Rattus concolor* group. In the recent scrub typhus report (Philip and Kohls, 1945) the name *Rattus concolor browni* was used and will serve in a general way, but *browni* is a Duke of York–New Britain race, and David Johnson and I feel that the Vogelkop race name could be used for that area, i.e., *Rattus concolor manoquarius.*" This rat was the *Rattus concolor browni* reported by Kohls et al (1945) as being taken in Area E, Sansapor. In addition, at least two other species of rat were trapped. There was a large coarse-haired species which was probably the Vogelkop representative of *Rattus ringens ratticolor,* and there was also an undetermined species of the genus *Melomys.*

Table 1 presents information concerning the rats and mites taken in the three environments at Sansapor. Data on one bandicoot is included. Six species of mites were found on rats. Kohls et al (1945) had reported four of these when they referred to mites in Area E in a discussion of tsutsugamushi fever in New Guinea and adjacent islands. It may be noted that *Trombicula deliensis* (syn. *T. walchi*), a proven vector, was taken on rats from all environments and was the most common mite found on rats. *Walchia glabrum*[3] was also prevalent and seemed to have a

TABLE 1.—*Data on trapped rats with their associated chigger populations**

Habitat	Number traps	Days trapped	No. days rats caught	No. rats caught	No. rats with mites	Trombicula deliensis	Walchia glabrum	Schöngastia blestowei	Schöngastia schüffneri	Ascoschöngastia indica	Ascoschöngastia impar	Not identified	Total mites	Mites per rat
Native garden														
Uncleared	9	49	18	31	26	116	200	72	6	..	14	316	724	23.4
Partially cleared	1	13	5	7	7	58	...	..	28	..	6	135	227	32.4
Rain forest														
Cleared—adjacent to grass	3	27	5	6	3	18	...	..	..	..	..	3	21	3.5
Cleared—not adjacent to grass	3	31	2	2	2	1	...	..	..	..	..	...·	1	0.5
Uncleared—not adjacent to beach	3	22	2	3	2	33	44	..	..	..	.·	13	90	30.0
Uncleared—adjacent to beach	3	15	2	3	3†	·16	52	..·	6	4	19	42	139	46.0
Beach														
Uncleared	1	7	3	7	7	108	4	..	..	10	1	375	502	72.0
Cleared—adjacent to grass	1	12	3	5	2	48	...	1	..	..	..	...	49	9.8
Cleared—not adjacent to grass	3	20	2	4	2	3	.·..	1	..	..	..	...	4	1.0

* The species of trombiculiid mites found in New Guinea have been the subject of considerable study during the war years. As a result of this work, names have been changed and placed in synonymy. Identifications for this report were made by the author (see Womersley, 1943, 1944). A representative sample was sent to Mr. Womersley for species confirmation. The nomenclature used in this paper is the result of recent correspondence with both Mr. Womersley and Dr. G. M. Kohls. The specific and generic names may still be subject to change, but it is believed that they represent the best ones now available. Wherever possible, synonyms have been included so that some confusion may be avoided.

† Includes one bandicoot.

[3] Kohls et al (1945) reported this as *W. disparunguis.* Correspondence with Mr. Womersley and Dr. Kohls indicate it is *W. glabrum.* All specimens examined by the author had bisetose third coxae.

general distribution.   The two species of *Ascoschöngastia* (syn. *Neoschöngastia:* See Ewing, 1946), *A. impar* and *A. indica,* appeared to be limited to grassy spots and beach area.   Although these two species were found in grassy area and in forest adjacent to beach, none were recorded in areas which were separated from the beach or grass by any considerable distance.   The specimens of *Schöngastia schüffneri* (syn. *S. pusilla*) were taken exclusively from the groin region of rats which belonged to the *Rattus concolor* group.   *S. blestowei* was common in grassy areas and was found in cleared beach sections, but was not taken in rain forest.

The majority of the Sansapor coastal sector was covered with virgin rain forest composed of large trees which yielded a dense shade.   The forest was generally quite open and little undergrowth was present.   As previously reported (Griffiths, 1945) tsutsugamushi fever did not appear to originate in this environment.   The three traps located in uncleared rain forest yielded only three rats.   Two of these belonged to the *concolor* group and these had populations composed about equally of *Trombicula deliensis* and *Walchia glabrum.*   Ninety mites were taken in all.   On another occasion an unidentified rat[4] captured in virgin rain forest had 33 *T. deliensis* and one *W. glabrum* in the sample which the author had for identification.   It will be noted that few rats were taken in rain forest.   The presence of *T. deliensis* on the rats would seem to indicate that tsutsugamushi fever might be contracted there.   In general it appeared that fewer rats were trapped per night and that they possessed fewer mites than was the case in grassy or beach areas.   It appeared quite probable that the low incidence of both rats and mites was the primary explanation for the fact that no tsutsugamushi cases could be traced to infection from forest area.   It should be noted (See Table 1) that the rats from cleared forest areas supported the same species of mites as those from the uncleared ones, but that total populations were considerably reduced.

Larval mites were recovered in some quantity from five different soldiers, all of whom were infested while working in uncleared rain forest.   All specimens taken in this manner were found to be *Trombicula wichmanni* (syn. *T. buloloensis:* See Philip and Woodward, 1946).   One additional specimen which was picked up on soil in rain forest was found to be a member of the same species.   This species was not found on any of the rats which were collected.

At the margin of beach and rain forest, grass and vines were found in abundance. These covered the ground and offered favorable cover for both rats and mites. Ninety cases of tsutsugamushi fever with four instances of mortality were known to have originated here.   Only one trap was located in a beach margin which had remained uncleared.   Rats were easily taken there, and they possessed large mite infestations.   About 90% of these were *Trombicula deliensis*.   Traps set in areas of beach margin which were inhabited by troops and which had been thoroughly cleared of all undergrowth and grass yielded only four rats.   A total of four mites were found on them.   Three were *Trombicula deliensis* and one was *Walchia glabrum*.   In cleared beach margin which was adjacent to grass, rats were fairly plentiful.   However, they possessed low mite populations which were preponderantly *T. deliensis*.   Both here and in rain forest, clearing appeared to have reduced the mite populations on rats.   This is in line with epidemiological evidence (Griffiths, 1945) that thorough clearing prevented further tsutsugamushi fever incidence in those organizations which had unfortunately camped in infectious areas.

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[4] Specimens furnished by Capt. Carl E. Mohr, Sn.C., AUS.

Scattered along the coast were areas which had been cleared at some previous time by natives and used either as village sites or as locations for gardens. Some of these areas such as the Sansapor village and Mar village (See Griffiths, 1945, for map) contained coconut palm groves and fairly extensive kapok tree plantings. All were characterized at the time of American occupation by a dense grass ground cover and scattered clumps of banana trees. Grass samples from behind Mar village at the mouth of the Wewe river were sent to Australia for identification.[5] They proved to be *Paspalum conjugatum* (family GRAMINEAE), *Eleusine indica* (family GRAMINEAE), *Phragmites communis* (family GRAMINEAE), and *Cyperus iria* (family CYPERACEAE). *Paspalum conjugatum* formed the bulk of the grass present. However, *E. indica* and *C. iria* were also common and were scattered throughout the area. *P. communis* was found only near the river margin and would appear to have no relation to tsutsugamushi incidence. The grass species found at Sansapor village and in other garden and village areas were essentially the same as those reported above. This type of environment produced more than 500 cases of tsutsugamushi fever. Rats of the *concolor* group were plentiful and mites were abundant. Boot collections by the author in the Wewe river area yielded a few specimens of *Schöngastia blestowei*, but no other species were taken in this manner. Kohls et al (1945) reported *S. blestowei* in boot collections at Sansapor and also mentioned *S. schüffneri* on man, but no reference to the latter's environmental source was given.

Although it was often possible to trace the source of infection to not only a general environmental locality, but also to specific spots within that environment, it was sometimes difficult to understand why troops living in apparently identical environments often exhibited marked differences in tsutsugamushi incidences. An excellent example of this was presented by the first battalion of one infantry regiment. It moved into the grassy garden area behind Mar village near the mouth of the Wewe river on August 1, 1944. The approximate disposition of troops by company is shown in Fig. 1. This area was the one from which the previously mentioned grass samples were obtained. Rats were abundant here and they were uniformly infested with heavy chigger populations. Headquarters Company, "D" Company, and "A" Company remained in the area only three days. On August 4, they moved east of the sawmill into a rain forest area. There were no cases of tsutsugamushi fever in "D" Company, and the only casualty in Headquarters Company was a soldier who was apparently infected while on detached service with another unit. This was in spite of the fact that these companies were in apparently identical environments to those of the other companies. Nine cases occurred in "A" Company. The first was on August 11, ten days after arrival at the Wewe river and six days after the unit had moved from the grassy area to a rain forest location. The last case in this company was reported on August 19, 15 days after leaving the infectious area. Fig. 2 shows the casualty rates for "A," "B," and "C" Companies.

· Both "B" and "C" Companies suffered heavily with 63 and 33 casualties respectively. Both remained near the river until August 14. The first case appeared in "C" Company only seven days after arrival at the river. Although this company had originally held a position beside "B" in a grassy area, it moved on August 4 and held a perimeter as shown in Fig. 1. Most of this was in rain forest. This would

appear to satisfactorily explain the decrease in casualty rate following the initially high incidence. All of "B" Company remained encamped in grassy territory until August 14. This prolonged exposure resulted in a high tsutsugamushi incidence. Thus, cases in this company started on August 11 and continued until 16 days after leaving the infectious area. Thus, Fig. 2 shows that incubation periods varied between seven and 16 days which are similar to the ones previously reported (Griffiths, 1945).

Thus, all five of the companies were in grassy areas which were apparently very satisfactory for contracting tsutsugamushi fever for at least several days. However, Headquarters and "D" Companies did not have any cases at all. This was in spite of the fact that between August 1 and August 4 they were in apparently identical situations with the other companies. Close examination of the epidemiology revealed
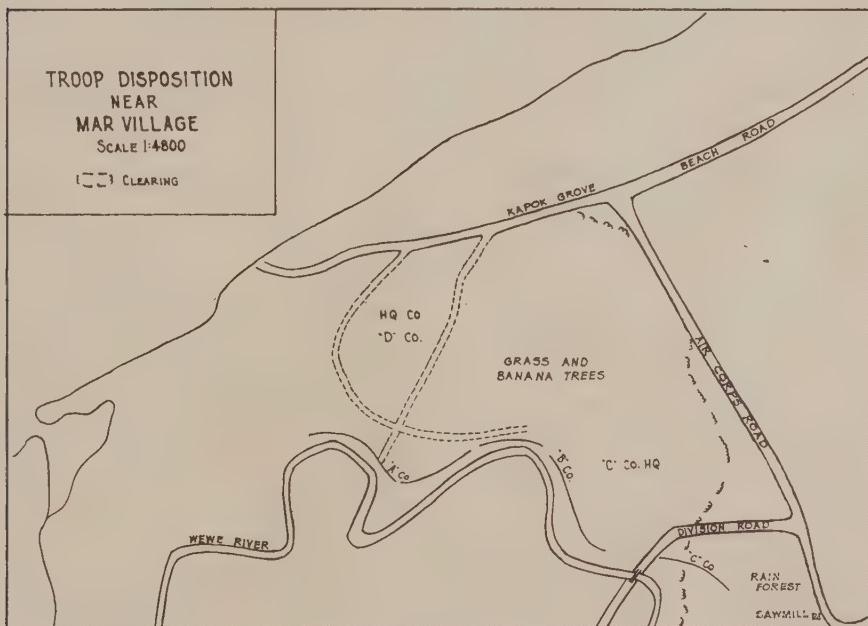
FIG. 1.

the apparent fact that infection often occurred locally within a larger grassy area. Thus, the members of one squad might almost all be infected. A possible explanation for this phenomenon is that a mature female mite infected with the disease would probably lay her eggs within a relatively small area. The infected larvae of the next generation would thus be found within a small radius. This condition would satisfactorily explain the very high incidence recorded for groups as small as a squad which had lived or slept within a confined grassy area.

In November of 1944, the author made a short trip to the United States base at the southern tip of Morotai in the Halmahera Island group. At that time no cases of tsutsugamushi fever could be traced with absolute certainty to that island. Traps were placed in grassy coconut groves which offered environments similar to known infective areas at Sansapor. Small unidentified rats were very readily captured.

Although a total of nine rats were taken, only one mite, *Walchia glabrum,* was found. The scarcity of mites would appear to be correlated with the absence of tsutsugamushi fever in the area.
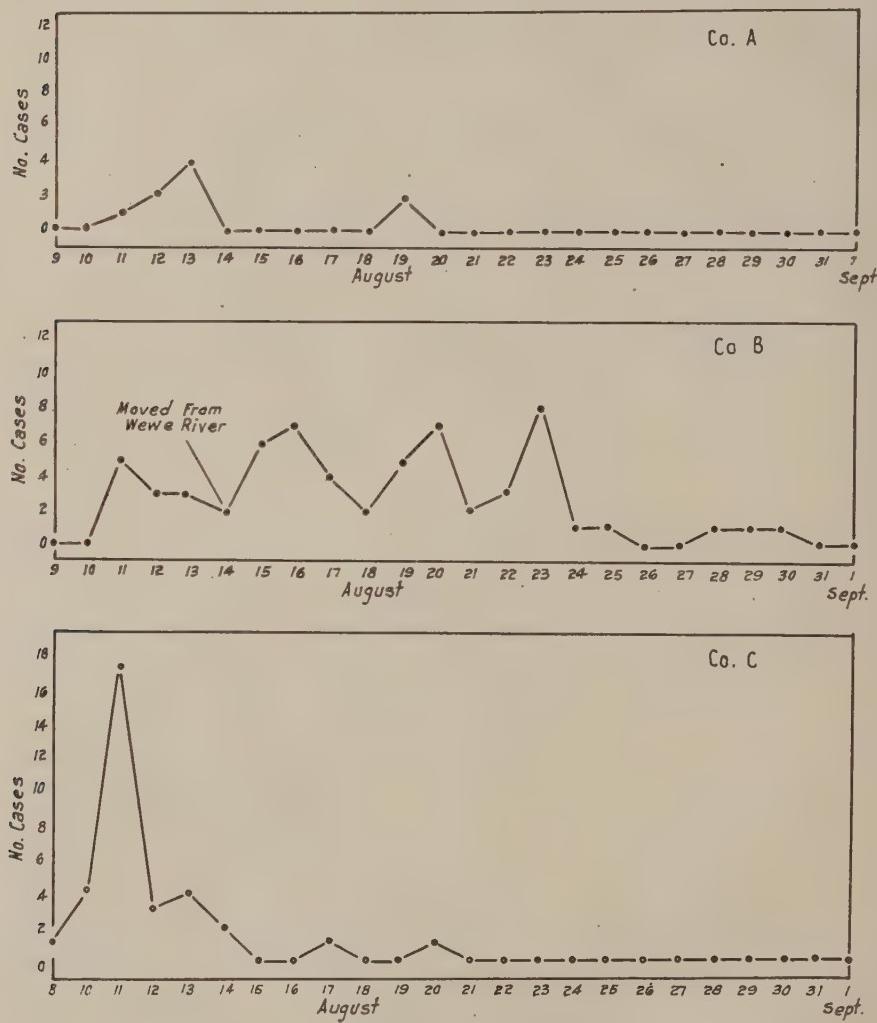

Fig. 2. Scrub typhus cases in 1st Bn.

SUMMARY

Rats with their attached mite populations were trapped in various environments at Sansapor, Dutch New Guinea. An attempt to correlate these findings with known tsutsugamushi incidence was made. Six species of mites were removed from rats. The most common were *Trombicula deliensis,* a proven tsutsuganiushi vector, and *Walchia glabrum.* These were found in all environments. *Schöngastia blestowei* was taken in boot collections, but only *Trombicula wichmanni* was actually found at-

tached to man. This latter species was not found on rats. Although the number of rats captured in cleared areas was not materially reduced, rats taken there had fewer chiggers than those from uncleared portions of the base. This observation agreed with the findings that tsutsugamushi fever could be controlled by carefully clearing an area. The peculiarities of the tsutsugamushi incidence in one battalion are discussed.

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HOPLOPLEURA OENOMYDIS FERRIS, A LOUSE FOUND ON DOMESTIC RATS IN THE UNITED STATES[1]

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Hoplopleura oenomydis Ferris (1921) was described on a basis of specimens taken from *Oenomys hypoxanthus bacchante* from British East Africa. Along with the type series, other specimens were recorded from *Dasymys incomptus helukus* and *Thamnomys surdaster polionopus,* British East Africa. Additional specimens were recorded from *Limnomys mearnsi* and *Rattus calcis,* Philippine Islands.

Ewing (1924) described as *Hoplopleura pacifica* similar specimens taken from *Rattus hawaiiensis* in the Hawaiian Islands. Other specimens were recorded from *Rattus* sp. (? Fanning, Line Islands) ; *Rattus* sp. (? Rosa Island, Samoa) ; *R. raveni* (? Celebes) ; *R. raveni eurous* (? Celebes) ; *R. surdus* (? Sumatra) ; and *R. concolor* (? Siam). Ewing also indicated that he considered the specimens from *Rattus calcis* from the Philippines to be representatives of an undescribed species.

Ferris (1932) recorded *H. oenomydis* from *Rattus* sp., Marquesas ; from *Rattus rattus diardi,* Federated Malay States ; from *Mus* sp., Sumatra ; and from *Rattus norvegicus,* Australia. At the same time Ferris considered *H. pacifica* to be a synonym of *oenomydis,* and this conclusion was later (1935) supported by an examination of specimens from rats taken in the Hawaiian Islands.

H. oenomydis is thus known to be a widely distributed species which may be found on a number of murine rodents. The wide geographical distribution of this ectoparasite may be readily explained by the present evidence that it has been brought to the United States by domestic rats and that large populations may be maintained by these rodents.

The writer first observed *H. oenomydis* from *Rattus norvegicus* taken in Jacksonville, Florida. During the period December 1945 to December 1946, there were 601 live rats trapped in Jacksonville, of which 101 rats were found to be positive for this louse. A total of 4,172 specimens of *H. oenomydis* were collected during this period. The largest number of specimens taken from an individual rat was 1,939 (372 males, 717 females, and 850 nymphs).

A study of the rat ectoparasites in Jacksonville, Florida, was reported by Rumreich and Wynn (1945) from collections made during the year 1934. According to these authors, 35.9 per cent of the lice collected represented *Hoplopleura hirsuta.* The approximation of this percentage to that found by the writer in Jacksonville in 1946, for *H. oenomydis,* together with a complete lack of *H. hirsuta* during the latter period, presents strong evidence that a misidentification was made during the earlier study.

Three Norway rats were collected in Tampa, Florida, during 1946, from which 78 specimens of *H. oenomydis* were combed. None of these lice, however, were collected from rats taken in connection with projects operating in Miami, Bartow, St. Petersburg, Tarpon Springs, Dunedin, or Pensacola, Florida.

Collections of *H. oenomydis* have been made in other States. This species has been found commonly on *R. norvegicus* taken in Atlanta, Georgia, where as many as an estimated 5,000 specimens have been taken from a single rat. A number of specimens also have been collected in DeKalb County and Crisp County, Georgia. Other specimens have been seen from domestic rats trapped in Nashville, Tennessee; Mobile, Alabama; Galveston, Houston, and Harris County, Texas; and Ames, Iowa.

The writer concurs with Professor Ferris in regarding conspecific specimens from British East Africa, the Hawaiian Islands, Philippines, Marquesas, Federated Malay States, Sumatra, and Australia. The types and other specimens from these localities in the Ferris collection have been examined by the writer and compared with material from the United States. The figure of the pleural plates given with the original description in Ferris's excellent monograph is inaccurate, but the drawings of these plates which were presented by Ferris (1932) will serve for ready recognition of the species. The variation which is there noted is covered by the large series at hand from the United States.

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MYONYSSUS JAMESONI, A NEW LIPONYSSID MITE (ACARINA: LAELAPTIDAE) FROM BLARINA BREVICAUDA (SAY)

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Myonyssus jamesoni is the third species to be described in this genus of ecto-parasitic mites belonging to the Liponyssinae, family Laelaptidae. The genus may be distinguished by the large shield covering the dorsum in both sexes and by the extremely large, wide anal plate and the posteriorly expanded genitoventral plate of the female. A short review of the genus is given.

Myonyssus Tiraboschi, 1904

Myonyssus Tiraboschi, 1904, Archives de Parasitologie VII (2) : 337.

Type.—*Myonyssus decumani* Tiraboschi.

Myonyssus decumani Tiraboschi

Myonyssus decumani Tiraboschi, 1904, Archives de Parasitologie VII (2) : 337, 338, fig. 68; Hirst, 1916, Jour. Zool. Res. 1 : 65–66, figs. 2, 3; Vitzthum, 1929, Die Tierwelt Mitteleuropas, Acari 3 (3) : 24; Radford, 1942, Parasitology 35 (1, 2) : 63.

Hosts.—In nests of and on *Rattus norvegicus* and *Mus musculus,* Europe.

Myonyssus gigas (Oudemans)

Liponyssus gigas Oudemans, 1912, Ent. Ber. 3 (64) : 231; (1913) 1914, Archiv für Naturgeschichte A (9) : 84–91, figs. 303–317.
Myonyssus gigas (Oudemans), Hirst, 1916, Jour. Zool. Res. 1 : 66–67, fig. 4; Vitzthum, 1929, Die Tierwelt Mitteleuropas, Acari 3 (3) : 24; Radford, 1942, Parasitology 35 (1, 2) : 63.

Hosts.—In nests of and on *Talpa europaea, Apodemus flavicollis* and *A. sylvaticus,* Europe.

Key to the Species

1. Anal plate of female about three times as wide as long, anterior margin distinctly concave, no setae on plate anterior to anal opening; setae on ventral plate of male of medium length **2**

   Anal plate of female about one and a half times as wide as long, anterior margin almost straight, occasionally a seta on plate anterior to anal opening (Fig. 8); setae on ventral plate of male extremely long .... *decumani* Tiraboschi

2. Genitoventral plate of female rounded posteriorly (Fig. 6), the three pairs of posterior body setae of approximately equal length (Fig. 7); setae laterad of genitoventral plate of male of approximately equal size, dorsal body setae of male relatively long .... *gigas* (Oudemans)

   Genitoventral plate of female not rounded posteriorly but irregular, the inner pair of the posterior setae much longer than the other two pairs; setae laterad of the genitoventral plate of male much shorter toward body margin, dorsal body setae of male relatively short .... *jamesoni,* new species

Myonyssus jamesoni, new species

Female (Figs. 1–3) : Palpus rather short, extending slightly beyond the femur of leg I; terminal segment small, papilliform, with several subequal, short, sharply pointed terminal setae and a large bifurcate subbasal curved spine situated internally. Body rather stout, broadest behind posterior legs ; shoulder region moderately developed. Chelicerae very slender, styliform, chelae very slender and sharp. Chelicerae in *jamesoni* appear to be intermediate between the common type in Liponyssinae and those found in Dermanyssinae. Dorsal shield with reticulate sculpturing as shown on anterior portion of Fig. 3 ; majority of dorsal setae very short but a few of the marginal setae much longer ; inner posterior setae long, about twice as long as the outer pair below the round posterior marginal sense organ and slightly longer than the long posterior ventral setae shown in Fig. 1. Jugularia absent but a differentiated jugular area present which extends for the entire width of the sternal plate as a reticulate band. Sternal shield reticulate, with convex, sometimes sharply so, anterior margin, and slightly concave posterior margin ; shape of sternal shield may vary somewhat. Anterior pair of sternal setae situated approximate to but not on anterior margin of plate ; each seta of second pair situated slightly laterad to line joining anterior and posterior setae. Genitoventral plate reticulate, more or less angularly expanded behind coxae IV ; the shape may vary as shown in Fig. 2, and in some cases a small area posterior to the genitoventral shield was found to be sclerotized ; the number of setae on the genitoventral plate may vary from 16 to 20, all more or less arranged without pattern. Anal plate reticulate, almost three times as wide as long, with concave anterior margin ; the setae laterad of the anal opening short, that posterior to the opening almost twice as long as lateral setae (in *gigas* the posterior seta is only slightly longer than the others) ; in most cases there is a pair of setae on the posterior lateral margin of the anal plate (in 1 of 12 specimens examined this seta was situated just off the anal plate). The pair of long setae just laterad of the setae on the margin of the anal plate is shorter than the long pair on the posterior margin. The metapodal plate is longitudinal and elliptical. Legs stout, first and fourth pairs subequal and longer than others ; second pair somewhat stouter than others ; third pair about as long as second pair but not so stout. Coxa I bears an inner basal blunt tubercle and a pair of setae ; coxa II has a large anterior spine and a posterior setae ; coxa III bears a pair of strong setae ; coxa IV has a single seta. Length of body, not including rostrum, 972 μ; 615 μ wide.

Male (Figs. 4, 5) : Chelicerae stouter than in female ; fixed chela slender as in female but blunt pointed ; movable chela much stouter, somewhat flattened, tapering at apex but with a minute truncated point. Body with sides subparallel and shoulder regions poorly developed. Dorsal shield with reticulate sculpturing as shown on anterior lateral and central portion of Fig. 4 ; dorsal setae short, marginal setae much longer ; two pairs of posterior marginal setae very long ; the setae lying above the posterior marginal sense organ several times longer than in female. Ventral plate shaped and with reticulate pattern as shown in Fig. 5, with 43 setae, the greatest number between coxae IV and the anal opening, apparently arranged in a heterogenous grouping rather than in a pattern of transverse rows. Setae just laterad of ventral plate somewhat shorter than those on plate but about twice as long as those on lateral margins. Metapodal plate longitudinal, narrow with parallel sides ; a small plate between the metapodal plate and ventral plate. Legs similar to those of female. Coxa I with a pair of setae ; coxa II with a large anterior spine and a pair of strong anterior and posterior setae (the teeth behind the anterior spine are larger in *gigas* than in *jamesoni*) ; coxa III with a large anterior and posterior seta ; coxa IV with a single seta. Length of body, not including rostrum, 900 μ; width 542 μ.

Immature forms not known.

Type host: Blarina brevicauda (Say) (short-tailed shrew).

Type locality: Monroe County, Pa.

Type: Female ; Monroe County, Pa.; May 14, 1945; Francis Harper; United States National Museum No. 1754.

Paratypes: Female ; same data as type, but May 12, 1945. Five females; Welland County, Ontario, Canada ; August 30, 1946; E. W. Jameson. Female; same data, but August 28, 1946. Female; Moorestown, N. J.; April 16, 1933; G. R. Lunz, Jr. Female; Fairfield County, Ohio; April 23, 1935; Robert Goslin. Female; Nantucket, Mass.; June 13, 1936; C. N. Smith.

Remarks.—In addition to the type series we have examined the following: Two females; Greenbriar, Tenn.; April 18, 1931; R. L. Boke. One female; Welland County, Ontario, Canada; September 13, 1942. Two males; Welland County, Ontario, Canada; August 30, 1946; E. W. Jameson. Male; Ithaca, N. Y.; November 29, 1937; W. J. Hamilton, Jr.

Hirst (1916) described and figured the female of a species of *Myonyssus* which he regarded as *gigas* that was represented as having a sculptured dorsum with conspicuous longitudinal ridges or carinae between the sculptured areas. Oudemans does not mention such sculpturing or ridges in his description of *gigas*. We have not found them on any of the specimens of *jamesoni* or on any of the three lots of 16 specimens of *gigas* from Europe; we have not seen material of *decumani*.

EXPLANATION OF FIGURES

Myonyssus jamesoni, new species
    FIG. 1. Ventral view of female.
    FIG. 2. Genitoventral plate of female showing variation in shape.
    FIG. 3. Dorsal view of female.
    FIG. 4. Dorsal view of male.
    FIG. 5. Ventral view of male.

Myonyssus gigas (Oudemans)
    FIG. 6. Genitoventral plate of female.
    FIG. 7. Posterior setae of female.

Myonyssus decumani Tiraboschi
    FIG. 8. Anal plate of female (after Hirst, 1916).
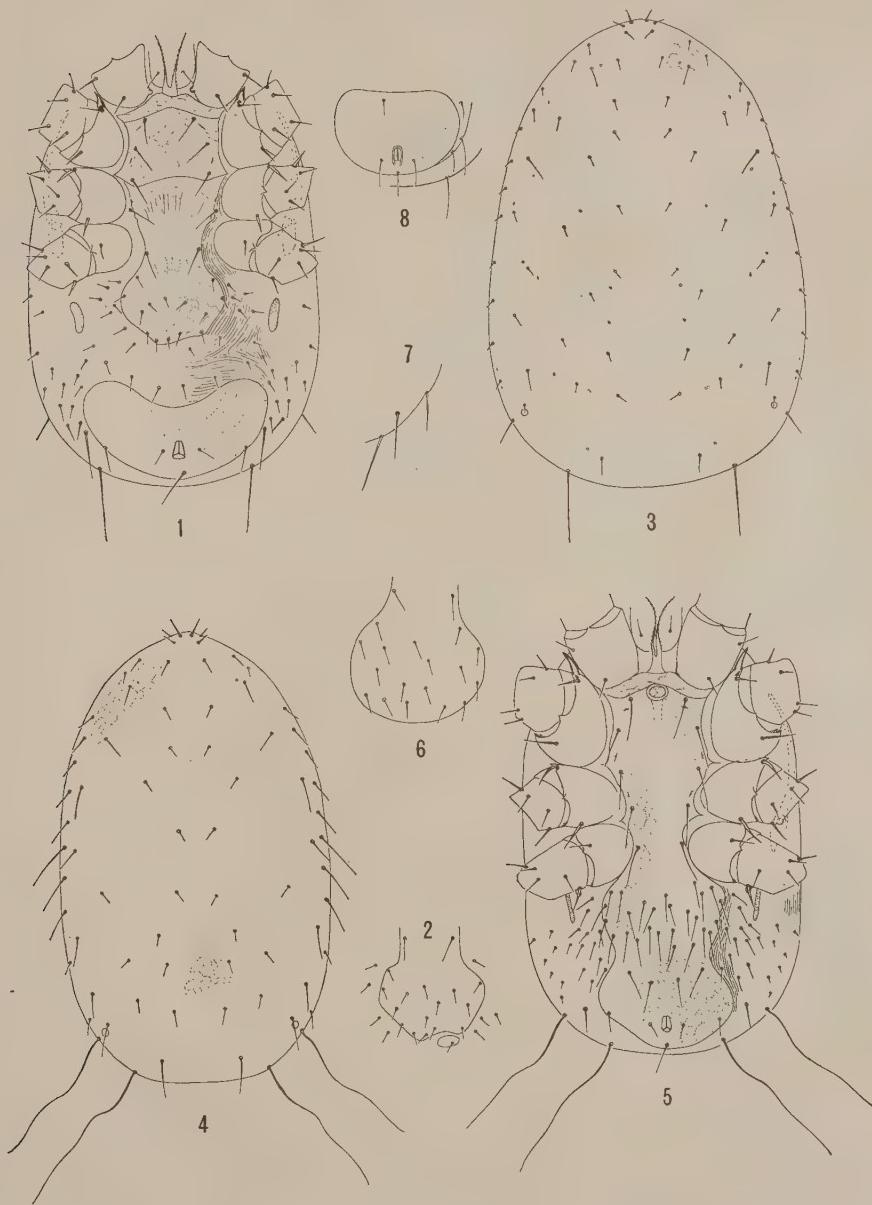
STUDIES ON NORTH AMERICAN CHIGGERS. 2. THE SUBFAMILIES AND *WOMERSIA STRANDTMANI* N. G., N. SP.

G. W. Wharton

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INTRODUCTION

The specimens to be described represent a new genus of chiggers that cannot be placed in an appropriate subfamily if Ewing's (1946) key to the subfamilies is used. Therefore it has been necessary to restudy Ewing's classification of the chiggers. Our present conception of the family and its genera is based almost entirely on Ewing's excellent work. With the advent of many new workers into the field, most of whom were introduced to the study of chiggers by Ewing himself, and with the subsequent increase in the body of information available; it is natural that new concepts should be developed.

Ewing (1929) established the subfamily Trombiculinae to include the chiggers. He defined the subfamily by listing the genera that comprised it. Later (1944) he raised the subfamily to familial rank, and separated the Trombiculidae from the Trombidiidae, the family in which the Trombiculinae had been placed, by characteristics of adult morphology and the habits of the larvae. Larvae that were parasitic on vertebrates were considered to be trombiculids, those parasitic on invertebrates were considered to be trombidiids. Unfortunately most trombiculids are known only from the larval stage, while many trombidiids are recognizable only as adults. It therefore seems desirable to define the Trombiculidae on the basis of larval morphology, and include in the family only those species whose larvae are consistent with the diagnosis of the family.

Trombiculidae Ewing 1944

Larval Diagnosis: Chelicerae with two segments; the basal segment stout and muscular the distal segment a sclerotized curved blade with or without projections called teeth. Palps with five segments: the basal segments are fused along the midline and have a median anterior laminar projection that extends beyond the basal segment of the chelicerae, and a pair of lateral wings or galeae that curl dorsad about the chelicerae and bear a seta on each side, each basal segment also bears a seta posterior to the junction with the palpal femur; the second palpal segment or femur bears a single seta; the third or genu bears a single seta; the fourth or tibia has three setae; one dorsal, one lateral, and one ventral and a terminal palpal claw; the fifth or tarsus articulates ventrally with the tibia and opposes the palpal claw in thumb-like fashion, it bears several setae (usually eight) the basal one of which is a striated sensory seta. The body is usually red in color but may be almost colorless; it bears: a dorsal plate or scutum at the level of the anterior two pairs of legs, usually two pairs of eyes that flank the scutum, several rows of dorsal setae, several rows of ventral setae, occasionally a posterior plate or a posterior group of specialized setae, a ventral anus, three pairs of legs, an urstigma or sclerotized pit associated with the posterior distal angle of coxa I, and at times a pair of tracheal trunks that open through stigmata in the region of the gnathosoma. The scutum bears from three to six marginal scutal setae or infrequently more and a pair of pseudostigmata from which the sensillae or pseudostigmatic organs arise. The legs are composed of six segments if the femur is undivided and of seven if the femur consists of a basifemur and telofemur.

If the above diagnosis is accepted three of the four subfamilies included in the family by Ewing (1946) will remain while the Hemitrombiculinae will be excluded.

WHARTON—NORTH AMERICAN CHIGGERS

Some modification of Ewing's interpretation of the remaining three subfamilies seems desirable and the erection of a new subfamily to accommodate the new genus to be described is necessary.

TROMBICULINAE Ewing 1929

Ewing separated the Trombiculinae from the other subfamilies by the presence on the scutum of an anterior median seta. While this characteristic is important, and while all of the trombiculinids have an anterior median seta, two genera (*Odontacarus* and the new genus to be described here) are not typical trombiculinids nor are they members of the same subfamily. The following diagnosis includes characteristics that are found in all members of the subfamily and any species whose larvae do not exhibit them should not be included.

Larval Diagnosis: Trombiculids whose larvae have a median scutal seta, seven segments in all legs, at least four sternal setae, no median anterior projection on the scutum, and no stigmata or tracheal trunks.

LEEUWENHOEKIINAE Womersley 1944

Womersley (1945) raised his subfamily to the rank of family. Ewing (1946) did not accept this. The subfamily LEEUWENHOEKIINAE should contain the genera *Odontacarus* Ewing 1929 and *Chatia* Brennan 1946; as well as the genera included in the group by Ewing (1946) with the exception of *Apolonia* Torres and Braga 1939.

Larval Diagnosis: Trombiculids whose larvae have six segments in all legs, no more than two sternal setae and two setae on coxa I. They frequently have paired submedian anterior scutal setae, an anterior median projection of the scutum, and stigmata with tracheae.

WALCHIINAE, Ewing 1946

This subfamily has been described by Ewing (1946) on the basis of larval morphology. To this diagnosis might be added these characteristics: Leg I with seven segments, legs II and III with six segments, with at least four sternal setae and only one seta on coxa I. No change in the list of included genera as given by Ewing is recommended.

APOLONIINAE New Subfamily

Larval Diagnosis: Trombiculids with seven segments on all legs, one seta on coxa I, an anterior-median projection on the scutum, stigmata and tracheae present, numerous ventral setae between the posterior coxae, and the posterior lateral scutal setae not on the scutum.

Genera: Type *Apolonia* Torres and Braga 1939, and *Womersia* n.g.

KEY TO THE SUBFAMILIES OF TROMBICULIDAE

1. (2) All legs with six segments, two setae on coxa I.   LEEUWENHOEKIINAE.
2. (1) First pair of legs with seven segments.   3.
3. (3) Legs two and three with six segments.   WALCHIINAE.
4. (3) Legs two and three with seven segments.   5.
5. (6) With stigmata and tracheae.   APOLONIINAE.
6. (7) Stigmata and tracheae absent.   TROMBICULINAE.

Womersia n. g.
Type: *W. strandtmani* n. sp.

Larval Diagnosis: Apoloninids with an anterior median seta, two pairs of sternal setae, and filiform sensillae. Nymphs and adults unknown.

This genus is named in honor of the Australian acarologist H. Womersley whose work on chiggers of the Austro-Malayan and Oriental Regions greatly facilitated research on tsutsugamushi disease during the recent war.

Womersia strandtmani n. sp.

Fig. 1

This species is named in honor of R. W. Strandtman of the University of Texas Medical School who collected the larvae to be described.

Body: Elongated oval in shape with a constriction posterior to the third pair of legs, 590 microns long by 260 microns wide. A pair of eyes with well developed corneas on each side of the body at the level of the scutum. Striae well developed over the entire body.
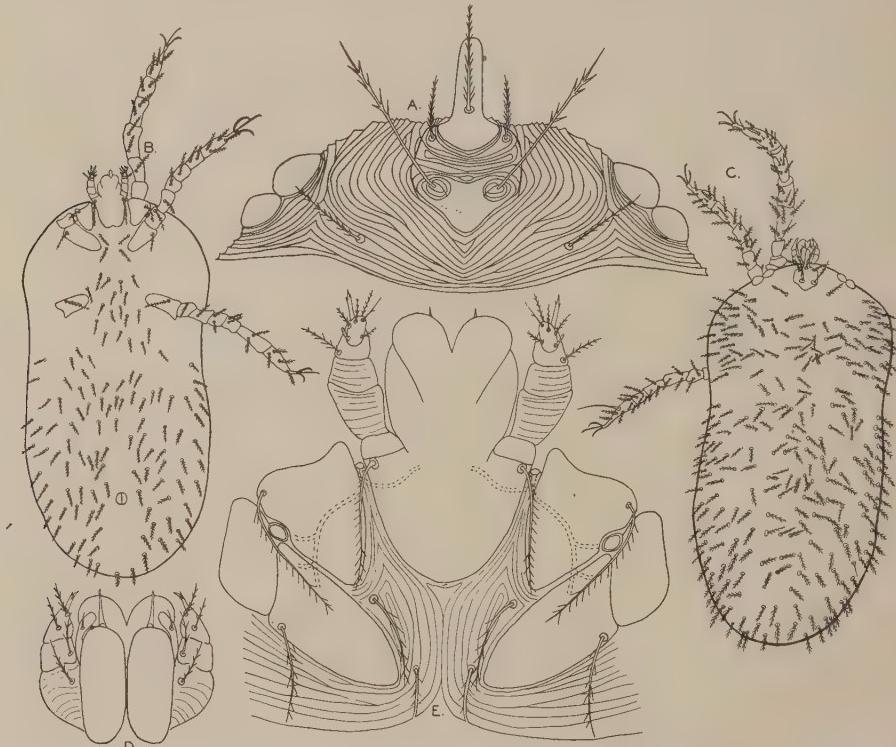
FIG. 1. *Womersia strandtmani:* A, scutum; B, ventral view; C, dorsal view; D, dorsal view of gnathosoma; E, ventral view of gnathosoma and propodasoma.

Gnathosoma: Chelicerae with evenly rounded elongated basal segments and small sharply pointed apical segments. Palpal segment 1 with a long feathered seta on its ventral surface, palpal segment 2 with a short feathered seta; a feathered seta with three or four barbs on palpal segment 3; dorsal, lateral, and ventral setae on palpal segment 4 with several barbs, palpal claw with two pointed lateral elements and a larger pointed median prong; palpal segment 5 with a basal striated sensory setae, 1 feathered seta at the apex with one or two barbs and 4 feathered setae with several barbs; galeal setae small and nude. A pair of stigmata open between the gnathosoma and coxae I, one on each side. Each stigma leads into two main tracheal trunks, one of which extends medially while the other runs posteriorly and laterally.

Legs: Coxae I and II contiguous coxa III separated from coxa II by a distance equal to twice its length, a single feathered seta on each coxa. One long feathered seta on each trochanter.

Basifemur I with one feathered seta, basifemora II and III each with two feathered setae. Telofemur I with six, II with four, and III with three feathered setae. Genu I with four feathered setae and one pointed striated sensory seta, II with four and III with three feathered setae. Tibia I with eight feathered setae and one micro-sensory seta that is flanked by a pair of pointed striated sensory setae, II with six feathered setae and two pointed striated sensory setae, and III with only six feathered setae. All tarsi with from ten to twenty feathered setae and in addition tarsus I has a micro-sensory seta posterior to a blunt striated sensory seta, a dorsal pointed striated sensory seta, and a pointed striated sensory seta flanking the pretarsus, II has a micro-sensory seta lateral to a blunt striated sensory seta and III has a single whip like seta. All tarsi terminate in pretarsi that bear a pair of subequal lateral curved claws and a longer thinner median claw-like empodium. The urstigmen are situated between coxae I and II in an indentation of coxa I.

Scutum: Scutum reduced so that the posterior lateral scutal setae are far removed from the plate. Scutum imbedded beneath the cuticle so that only the sensillary area and anterior projecting näse are free from superficial cuticular striae. Scutal setae feathered. Sensillae that arise from the pseudostigmata filiform with short barbs along the distal half. The näse is 20 microns in length from the base of the anterior median seta to the tip. The Standard Data (Wharton 1946) follow:

AW	PW	SB	ASB	PSB	AP	AM	AL	PL	S
18	51	15	40	12	32	30	20	22	47
17	51	17	41	14	30	27	22	23	44

Setae: Dorsal setae all feathered and of uniform length, twenty microns; numerous arranged in indistinct rows in two groups one anterior to the constriction of the body the other posterior. The anterior group has about 64 setae while the posterior group has over 150 setae. Ventral setae similar to dorsal setae. Sternal setae, two pairs median to the anterior coxae. A group of 16 ventral setae between coxae III followed by about 100 more posterior setae. The anus is situated in the midst of the posterior ventral setae 90 microns from the posterior end of the body.

Material.—Two specimens collected by R. W. Strandtman 19 June 1946 at Galveston, Texas from a brown pelican *Pelecanus occidentalis.* The type will be deposited in the U. S. National Museum while the other will be retained at Duke University.

Diagnosis.—*Womersia strandtmani* is the only species in the genus and differs from its nearest relative *Apolonia tigipioensis* as indicated in the generic diagnosis.

DISCUSSION

The discovery of *W. strandtmani* has made it possible to gain a better understanding of the variation to be expected among the members of the TROMBICULIDAE. Ewing's (1946) restudy of *Odontocarus* revealed the fact that except for the presence of only a median seta the genus was typical of the LEEUWENHOEKIINAE. However since the specimens were so faded it was difficult to be sure of many of the morphological features and thus the genus was placed with the trombiculinids since the line of demarcation between the trombiculinids and leeuwenhoekinids established on the basis of the presence of median or submedian scutal setal held good in all other cases. The fact that *Apolonia* and *Womersia* are so closely related, and yet one has submedian scutal setae while the other has a median scutal seta indicates that this difference is not as fundamental as was originally suspected.

A search for more important differences was then undertaken and it was found that the number of segments in the legs was consistent as far as determined within each subfamily as reported above. Other differences were also found such as the presence of the anterior sternal setae of the leeuwenhoekinids on coxae I, the extension of the ventral setae between coxae III in the apoloninids, the absence of

stigmata tracheae, and näse in all of the trombiculinids and walchinids while they are present in many of the leeuwenhoekinids and all the apoloninids.

It is realized that the classification presented in this paper is based entirely on an incomplete study of larval morphology and so may be subject to radical change when more information, especially when data concerning the nymphs and adults are obtained. Such a classification invites criticism from other workers but it is hoped that they will find the system presented here at least a fit subject for subsequent revision.

SUMMARY

1. The family TROMBICULIDAE has been defined on the basis of larval morphology.

2. APOLONIINAE, a new subfamily, has been erected and includes the genera *Apolonia* Torres & Braga 1939 and *Womersia* n. g.

3. A key to the four subfamilies of the TROMBICULIDAE has been given.

4. A new monotypic genus, *Womersia,* has been described as well as *W. strandtmani* n. sp.

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RESEARCH NOTE

OBSERVATIONS ON THE EXCYSTATION OF *ENDAMOEBA HISTOLYTICA*

As reported by Rees (1942, Am. J. Trop. Med. **22**: 487–492) a micro-isolation technique has been used to obtain bacteria-free cysts of *Endamoeba histolytica* which have provided cultures of the amoebae with selected single species of bacteria. In the absence of bacteria, the cysts have failed to produce cultures of amoebae. Test tubes or Wassermann tubes were used for this work and it was the practice to isolate from 8 to 25 cysts per tube. This practice was based on tests which showed that larger numbers of cysts per tube could not be isolated without the carry over of some bacteria. It was not known whether excystation occurred in the bacteria free preparations because repeated examinations failed to disclose either cysts or amoebae. Therefore, the technique has been modified so that the cysts may be isolated in microculture tubes through the walls of which the organisms can be seen under the microscope at any time during an experiment.

The microculture tubes are miniature test tubes constructed from capillary glass tubing approximately 1½ mm in diameter, in which several kinds of egg media and various wholly liquid media were dispensed. In order to observe the cysts while transferring them from the pipette to the microculture tubes and during the incubation period, the tubes were immersed in water. Immediately after introduction of the cysts, the tubes were sealed in a microflame. Exchange of gases between the medium and the air and contamination with bacteria during the period of incubation were thereby prevented.

In the microcultures prepared without bacteria, excystation was observed in all media except Locke's solution. These observations suggest that some unknown organic substances occurring in the media facilitated excystation and that dextrose alone, which was the only organic substance in the Locke's solution, was inadequate for excystation. Observation of 800 microcultures containing a total of approximately 7,000 cysts showed that 17 per cent excysted in one series but that less than one per cent excysted in other series. Examination of the cysts showed that 25 to 75 per cent were tetranucleate and on this basis the rates of excystation were surprisingly low. Cysts from human stools showed no higher rates of excystation than did cysts from culture. Addition to the media of cysteine hydrochloride had no appreciable effect on the rate of excystation but, since methylene blue was decolorized in tubes without this reducing agent, its addition was not required to lower the oxygen tension.

Control cultures with organism *t* showed only slightly higher rates of excystation than did bacteria free cultures. It would appear, therefore, that optimum conditions for excystation may not have been provided by the growth of bacteria.

Some comparative tests with *Endamoeba coli* indicated that excystation occurred under the same experimental conditions and at the same comparative rates as observed for *E. histolytica*.— CHARLES W. REES, LUCY V. REARDON, AND FRANCES E. JONES, *Division of Tropical Diseases, National Institute of Health, Bethesda, Md.,* AND ANGUS M. GRIFFIN, *the George Washington University School of Medicine, Washington, D. C.*[1]

AMERICAN SOCIETY OF PARASITOLOGISTS
ANNOUNCEMENTS

Change of address of Secretary.

The members of the Society are hereby informed that, beginning July 1, 1947, the secretary will be located at the University of Arkansas, School of Medicine, Little Rock, Arkansas.

Respectfully,

JAMES T. CULBERTSON,

Secretary

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Treasurer